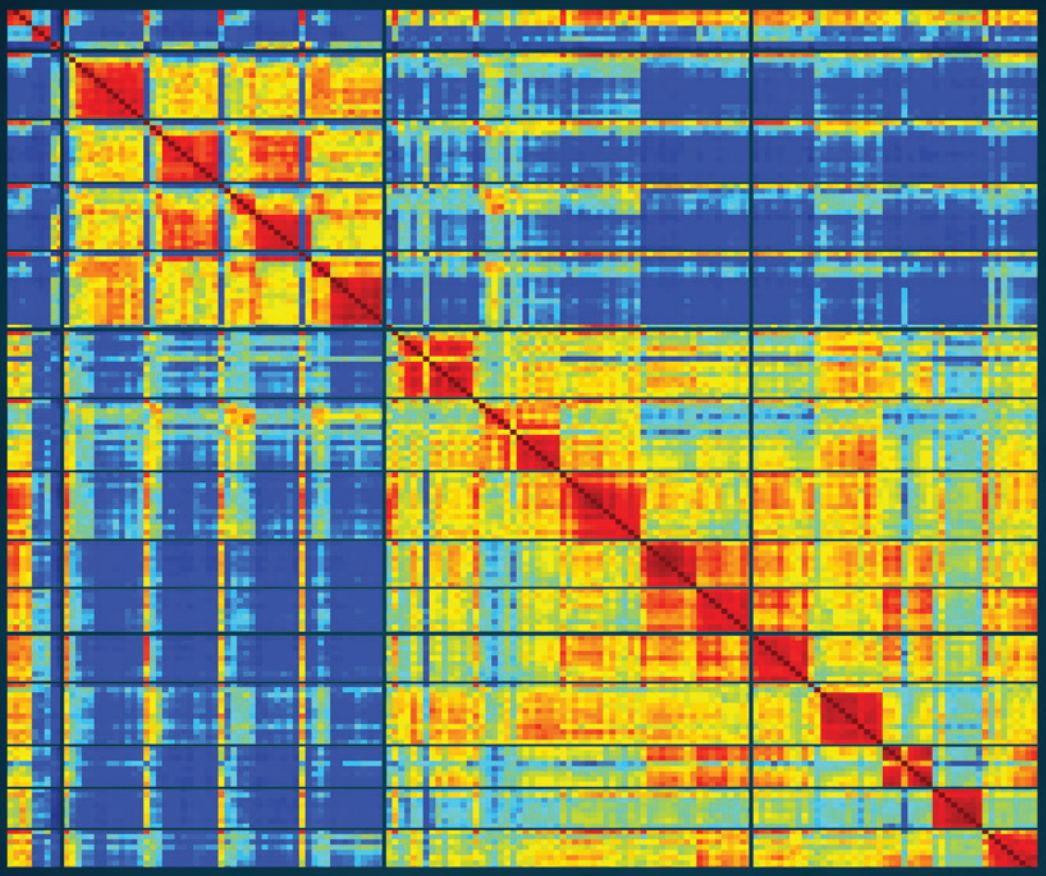
NIDDK

Recent Advances & Emerging Opportunities

March 2014



U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

ON THE COVER

The mammalian intestine hosts an enormous ecosystem of microorganisms. Mammals provide nutrients to their "guests" in exchange for essential metabolic functions that mammals cannot perform on their own. Microbial residents of the intestinal tract (microbiota), therefore, play important roles in mammalian digestion. However, a growing body of research has discovered links between the microbiota and diseases such as inflammatory bowel disease, obesity, and diabetes.

Recent research has shown that the composition of a person's microbiota can shift with diet and may even be a key component of weight loss resulting from gastric bypass surgery. The cover image illustrates the amazing diversity of bacteria that inhabit the mammalian gut and how these bacterial populations shift after gastric bypass surgery. Each block of color in the figure represents a stool sample taken from a mouse that received either sham surgery or a Roux-en-Y gastric bypass procedure. The mice that received sham surgery were further divided into groups that either ate as much as they wanted or were fed calorie-restricted diets to mimic the calorie restriction experienced by mice that received the gastric bypass procedure. Microbiota samples were taken at varying weeks post-surgery and their genetic material sequenced to identify the bacterial species in that mouse's gut at that point in time. These genetic "fingerprints" of the mouse's microbiome (the microbiota's collective genetic information) were then compared to microbiome fingerprints taken at other times and in other mice and plotted using a color key to indicate similarity. Each block is colored to indicate a range from "no relation" between the samples (dark blue) to "perfect correlation" (dark red). The dark red diagonal line indicates comparisons of each microbiome with that mouse's pre-operative microbiome.

The large amount of dark blue shown where the microbiomes of the mice from different treatment groups are compared (the bottom two-thirds of the first column) indicates that the mice that received gastric bypass surgery experienced a significant shift in what species of bacteria they hosted following the surgery, and that this shift was not entirely due to simple calorie restriction. This finding may inform future research on not only the effects of gastric bypass surgery but also on nutrition, obesity, and microbiota-related diseases.

Through continued support for research in this important area, the NIDDK aims to define the role of the microbiome in disease progression, with the hope of developing new therapeutic approaches to improve health.

Image provided by Dr. Lee M. Kaplan and Dr. Peter J. Turnbaugh, and from Liou AP, Paziuk M, Luevano JM Jr, Machineni S, Turnbaugh PJ, and Kaplan LM: Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 5: 178ra41, 2013. Reprinted with permission from AAAS.

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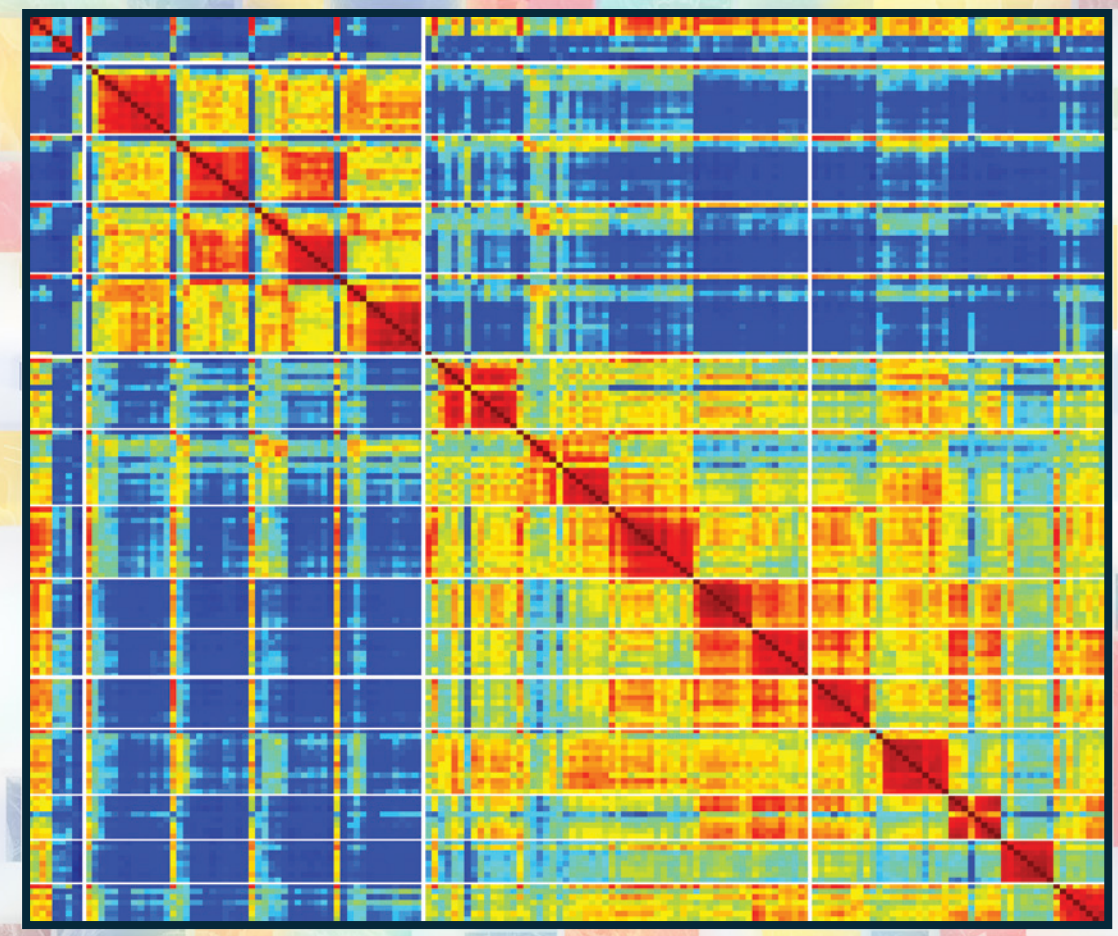

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CONTENTS

Message from the Director

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual report highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility that includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, such as cystic fibrosis; liver disease and other digestive diseases, such as inflammatory bowel disease; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases and conditions, such as interstitial cystitis/painful bladder syndrome; and hematologic diseases.

The 14th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Findings from a clinical trial that youth with type 2 diabetes face increased risks of heart, nerve, and eye complications
- Demonstration of the feasibility of a wearable, smartphone-based “artificial pancreas” prototype for management of type 1 diabetes
- Discovery of a new class of genes involved in maturation of pancreatic cells that produce insulin
- Evidence that a brief psychological and educational group therapy can reduce symptoms and improve quality of life for those with irritable bowel syndrome
- Discovery that a protein found in the brain regulates body weight and that its deficiency may be associated with obesity in humans
- New insights into the roles that bacteria in mammalian intestines (gut microbiota) play in the link between gut inflammation and cancer, in severe malnutrition, in metabolism and obesity, in the weight loss seen following gastric bypass surgery, and in blood pressure regulation
- Identification of a genetic mutation that causes a distinct form of hemolytic uremic syndrome, a condition marked by kidney damage and abnormal blood cell function, with implications for treatment strategies
- Finding that beverage choice is associated with progression or reduction of urinary tract problems
- Development of a new technique to deliver therapeutic drugs across the blood-brain barrier, which may lead to treatments for lysosomal storage disorders

This report also includes personal stories of patients. A doctor with type 1 diabetes describes his participation in a study aiming to determine why some people with the disease develop complications while others do not. A participant in a type 2 diabetes prevention study and his identical twin brother share their experiences with lifestyle changes to improve their health. A woman discusses the daily difficulties of living with fecal incontinence and shares her resolve to help others with this debilitating and often hidden condition.

The NIDDK is continuing efforts to ensure that knowledge gained from research advances it supports is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's education programs: the National Diabetes Education Program and the National Kidney Disease Education Program. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available to patients, health care providers, and the public both in printed format and on the NIDDK website. I invite you to visit us at *www.niddk.nih.gov*

This report reflects only a fraction of the immense body of NIDDK-funded research performed by basic scientists, clinical investigators, and patient volunteers. Moving forward, we remain committed to supporting these important areas of research and translating scientific discoveries into improvements in the health and quality of life of all people.

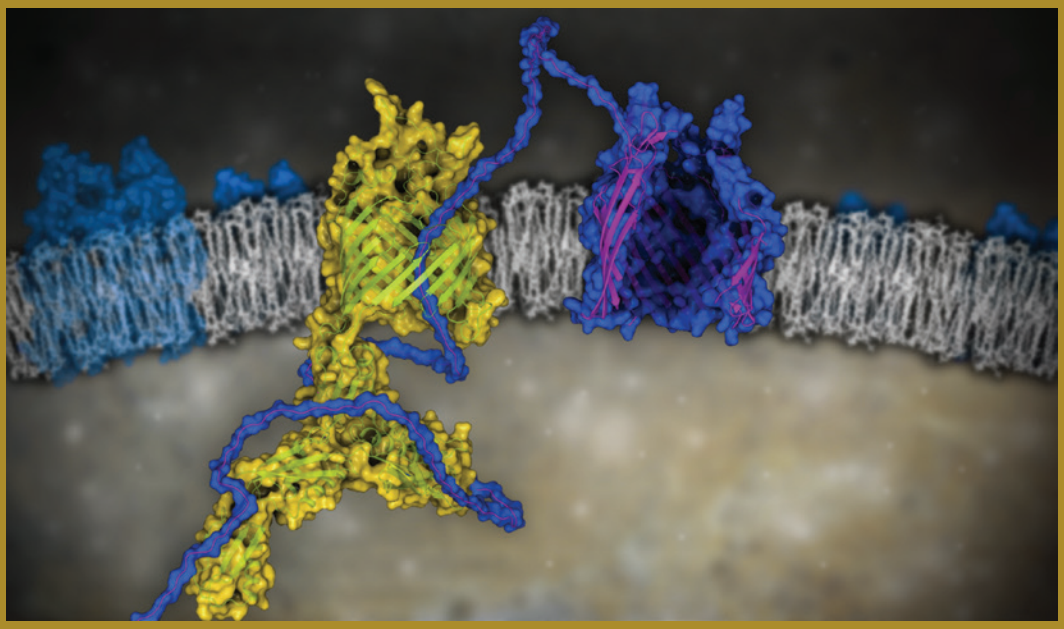
The efforts featured in this publication reflect the core mission of the NIDDK, including the Director's guiding principles:

- Maintain a vigorous investigator-initiated research portfolio
- Support pivotal clinical studies and trials
- Preserve a stable pool of talented new investigators
- Foster exceptional research training and mentoring opportunities
- Ensure knowledge dissemination through outreach and communications

More information on how NIDDK's activities support these core values can be found in the "NIDDK Funding Trends and Support of Core Values" section at the end of this report and on our website at *www.niddk.nih.gov*

Griffin P. Rodgers, M.D., M.A.C.P.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services

Scan the QR code with your mobile device to link to the NIDDK website at *www.niddk.nih.gov*



The surface of a bacterial cell is covered with a variety of proteins required for survival, including those that allow the cell to sense and interact with its environment, communicate with other cells, or evade the mammalian immune system. As described in this chapter, NIDDK researchers have determined the detailed atomic structure of the cellular machinery responsible for incorporating these essential proteins into the bacterial surface. Knowing the structure of the major assembly component (known as BamA, depicted in yellow/green) provides insight into how bacteria place proteins (blue/purple) at their surface and suggests new drug targets to combat infectious diseases.

Image provided by Dr. Nicholas Noinaj, Dr. Adam Kuszak, and Dr. Susan Buchanan, NIDDK.

Cross-Cutting Science

Medical advances are not always achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of new knowledge from years of scientific research. Insights into the fundamental biologic building blocks and processes of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our knowledge of disease and disease treatment can be traced to laboratory studies whose relevance to health could not have been fully known or appreciated at the time they were conducted. With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams join together to tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. The insights gained through this research can be expected to further scientific progress in many research areas, for today's discoveries may hold the seeds of tomorrow's cures.

Described in this chapter are several recent studies that illustrate the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. Also featured are several NIDDK programs that demonstrate the breadth and collaborative nature of the NIDDK's research efforts, including several clinical research studies which the NIDDK is uniquely positioned to support. These studies are, for example, evaluating new uses for medications, including generic drugs; testing behavioral or lifestyle changes as a therapeutic approach; or are large studies requiring significant investment. In many cases these studies benefited from partnerships with non-federal funders but were unlikely to have been undertaken solely by these other funding sources.

The NIDDK's trans-NIH and public-private collaborations have also led to the development of cutting-edge technology, such as microbial-resistant urinary catheters, more accurate drug toxicity screening tools, important artificial pancreas technologies, and state-of-the-art biomedical imaging.

Finally, the NIDDK is dedicated to delivering important health information to the public. Through disease-specific Information Clearinghouses and other online resources, the NIDDK offers a wide variety of informational materials to health care professionals, patients and their families, and others across the country and across the world.

AGING AND THE BRAIN

Inflammation in the Brain Links Obesity and Aging: Two studies further clarified the role of brain signaling in aging and metabolic disease. This research, done in mice, suggests that decreasing inflammation in the brain may reduce the signs of aging and age-related disease. Aging involves gradual functional decline and affects many tissues and organs. However, how aging determines lifespan is poorly understood, as is why the risk of certain diseases, like type 2 diabetes, increases with age. Inflammation plays a role in type 2 diabetes and other age-related diseases, but how inflammation influences aging is unclear. The brain has emerged as a potential regulator of aging and metabolism, and therefore understanding how metabolic disease and inflammation affect the brain—and *vice versa*—are key research goals.

Toward those goals, in a recent study researchers examined the role of the hypothalamus, a region of the

brain, in aging. The hypothalamus plays a key role in growth, reproduction, and metabolism. Given these important roles, researchers investigated whether the hypothalamus could also control aging and lifespan. Because previous research showed that inflammatory changes contribute to the development of metabolic syndrome, they focused on two inflammatory signaling proteins, IKK-β and NF-κB, which act together to trigger downstream inflammatory responses. The researchers found that NF-κB is more active in the hypothalamus of older mice than that of younger mice. To determine the effect of NF-κB on aging, researchers increased or decreased IKK-β/NF-κB activation in the hypothalamus of mice and evaluated age-related physiology. Mice with decreased NF-κB activation performed better on cognitive and strength tests, displayed more robust physical characteristics, and lived longer, while the mice with increased NF-κB activation performed poorly and had shorter lifespans than their normal counterparts. The effects of IKK-β/NF-κB on aging were further traced to gonadotropin-releasing hormone (GnRH), which is depleted as IKK-β/NF-κB activation increases. To test whether replenishing GnRH might reverse the effects of aging, researchers gave mice GnRH injections and found that this therapy prevented age-induced brain cell loss and reduced age-related skin, muscle, and cognitive degeneration. These results suggest that inflammatory responses in the hypothalamus orchestrate aging throughout the body and may give clues to how aging occurs and how lifespan can be increased.

In another study, several of the researchers who conducted the first study found a link between obesity-related inflammation and neurodegeneration, or loss of neuron function. They discovered that IKK-β/NF-κB signaling promoted metabolic disease by disrupting adult neural stem cells (NSCs) in the hypothalamus. NSCs are a type of stem cell; like other stem cells they are self-renewing and can develop into many different types of cells. NSCs, in particular, can become many different types of brain cells. The researchers sought to identify adult NSCs in the hypothalamus and to determine if disruption of normal NSC function plays a role in diseases such as obesity and diabetes. They found that in adult mice NSCs were particularly numerous in the hypothalamus, where they slowly produced new brain cells. A high-fat diet, however, reduced the NSCs' generation of new brain cells and prevented their development into different types of brain cells. The scientists found that increased IKK-β/NF-κB activation was responsible for these effects: reducing IKK-β/NF-κB activation promoted NSC proliferation and development into different brain cell types. Moreover, mice with increased IKK-β also developed glucose intolerance—a symptom of prediabetes and diabetes—and displayed overeating and weight gain, resulting in the onset of severe obesity. These results suggest that high-fat diets in mice are associated with inflammatory responses in the hypothalamus that can reduce the number and activity of neural stem cells, leading to neurodegeneration, obesity, and diabetes. Together, these findings in mice may provide a greater understanding of human aging and metabolism and lead to prevention or treatment strategies for obesity-related diseases, such as type 2 diabetes.

Zhang G, Li J, Purkayastha S, et al. Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH. Nature 497: 211-216, 2013.

Li J, Tang Y, and Cai D. IKKβ/NF-κB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. Nat Cell Biol 14: 999-1012, 2012.

New Insight into Alzheimer's Disease: Recent research reveals individual differences in brain tissue obtained from two patients with Alzheimer's disease who had distinct clinical histories and severity of brain damage. Alzheimer's disease is a progressive, irreversible brain disease that destroys memory and thinking skills. Many changes take place in the brain of a person with Alzheimer's disease. Some of these changes can be observed in brain tissue by using microscopy after death. A common abnormality evident in the brains of people who have died with the disorder is the amyloid plaque. The plaques consist predominantly of abnormal deposits of a protein fragment called beta-amyloid or β-amyloid, frequently abbreviated as Aβ. The molecular architecture of Aβ aggregates that develop in human brain tissue has not

been characterized in detail, but scientific findings to date suggest that structural variations may be biomedically important.

For the first time, scientists precisely characterized the molecular structures of Aβ fibrils that form in the brains of patients with Alzheimer's disease. Using sophisticated biophysical techniques, a single-length predominant fibril structure was recovered from each patient; however, the fibrils were structurally different from each other. These data suggest that brain fibrils appear first at a single site and then spread to other locations in the brain while retaining their respective identical structural signatures. The study further suggests that certain fibril structures may be more likely to cause and/or influence the severity of Alzheimer's disease. The development of imaging agents that specifically target individual fibril structures will improve the reliability and specificity of diagnosis.

Lu J-X, Qiang W, Yau W-M, Schwieters CD, Meredith SC, and Tycko R. Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. Cell 154: 1257-1268, 2013.

INSIGHTS INTO GENES, PROTEINS, CELLS, AND HEALTH

Increasing Mammalian Lifespan, One Gene at a Time: New research has shown that lowering the activity of a gene called *mTOR* dramatically increased the average lifespan of a group of mice and allowed them to maintain high function in several—although not all—tissue and organ systems that typically decline with age. Found in mammals, the *mTOR* gene has been implicated in such key processes as metabolism and aging. However, the mechanism of how *mTOR* reduction affects lifespan and tissue aging has not been fully understood. Previous studies showing that *mTOR* is involved in metabolic functions indicated that this gene may be connected with the increased lifespan associated with caloric restriction. In this study, researchers bred a group of mice to have genetic alterations that reduced their mTOR levels. Compared to normal mice, these mutant mice had an increased lifespan of around 20 percent, showed reduced signs of aging in many organ tissues, and, as they aged, retained more balance, grip strength, and cognitive abilities like learning and memory. Mice with reduced mTOR protein also appeared to have fewer malignant tumors than normal mice. These effects of mTOR reduction did not appear to result from altered metabolism, since mice with less mTOR had similar metabolic profiles compared to normal mice. However, these benefits did not come without a price: mTOR-deficient mice contracted more serious infections as they aged and had more pronounced bone deterioration than their normal counterparts. Together, these results suggest that tissue aging may occur on related, but separate, "clocks" or be subject to other distinct biologic regulation compared to aging of an entire organism. Further research into the mechanism of how mTOR reduction affects the body is ongoing and may guide future therapies to increase both lifespan and "health span" in humans.

Wu JJ, Liu J, Chen EB, et al. Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. Cell Reports 4: 1-8, 2013.

Finding Out How β-Barrels Are Built: Recent findings from the NIDDK Intramural Research Program are providing important insights about the formation of "β-barrels"—protein structures found in the exterior membranes of a large group of bacteria, many of which cause serious diseases. Most β-barrels act like pores, and many fulfill essential roles like allowing nutrients to get into the space between outer and inner membranes that is characteristic of Gram-negative bacteria. Chloroplasts—the structures inside plant cells where light is converted into chemical energy—and mitochondria—the structures in cells of both plants and animals that extract energy from a variety of fuel sources—share this double-membrane organization with Gram-negative bacteria, from which they are thought to have evolved, and also have β-barrel pores in the exterior membrane. The initial steps in the synthesis of proteins that contain β-barrels are the same as those of other proteins. The final step—insertion of the barrel portion into the outer membrane—is known to be accomplished by a group of other proteins called the "barrel-assembly machinery (BAM)" complex.

To obtain clues about how the BAM complex fulfills this role, the NIDDK scientists studied a critical component of this complex, the BamA protein, itself a β-barrel protein. They determined the three-dimensional structures of BamA from two different disease-causing bacteria and compared them to known structures of other β-barrel proteins. This approach helped them to identify unique features of BamA, and to provide likely explanations for their role in assembly of β-barrels.

The findings suggest that parts of BamA that are on the interior side of the membrane are able to bind to proteins requiring membrane insertion, to open a passage into BamA's β-barrel, and—working with another part of BamA that can move up and down in the barrel—in effect, to act like a ratchet to thread a portion of the new protein into the pore. The BamA pore itself has a feature that thins the bacterial membrane at a key point, and opens a fissure along the side of the BamA barrel, facilitating movement out of the pore and into the adjacent membrane.

The researchers hypothesize that this process repeats until all parts of the new protein needed to form a β-barrel have been properly arranged in the membrane, and the two proteins separate. The researchers also found that a simpler mechanism may be available for a subset of β-barrel proteins, where the inner, grappling/ratcheting portions of BamA might insert the new protein directly into the part of the membrane that is thinned by BamA, without actually passing through the interior of BamA's barrel. Further research will be needed to determine if these fascinating processes operate for β-barrel proteins in the outer membranes of other bacteria, or of mitochondria and chloroplasts.

Noinaj N, Kuszak AJ, Gumbart JC, et al. Structural insight into the biogenesis of β-barrel membrane proteins. Nature 501: 385-390, 2013.

Skin Strikes a Balance Between Growth and Integrity: A new study in mice has discovered that a molecule called sphingosine-1-phosphate (S1P) is an important—and sometimes vital—regulator of the skin's lifecycle. Skin cells are constantly growing, differentiating (producing specialized types of cells), dying, and being shed. Disruptions in this cycle can lead to diseases where skin is too thick or too thin. Mice lacking the gene for a protein that controls S1P had abnormally high S1P levels and stunted growth compared to normal mice when examined within a few days after birth. They also had serious skin abnormalities—the mutant animals had enhanced skin cell differentiation, which made their skin thicker than normal and prone to peeling and sloughing off. Many of these mice died by three weeks of age, and the few that survived to adulthood had skin abnormalities throughout their lives. Further analysis of the animals' skin cells demonstrated that increased S1P levels had wide-ranging effects, affecting the activity of over 700 genes, many of which are involved in skin cell differentiation. Therefore, this research suggests that S1P metabolism plays a key role in skin cell differentiation. These findings, if confirmed in humans, may lead to new treatments for skin diseases caused by abnormal skin cell differentiation, such as psoriasis.

Allende ML, Sipe LM, Tuymetova G, Wilson-Henjum KL, Chen W, and Proia RL. Sphingosine-1-phosphate phosphatase 1 regulates keratinocyte differentiation and epidermal homeostasis. J Biol Chem 288: 18381-18391, 2013.

Clinical Studies Highlight Importance of Public Biomedical Research Funding

The NIDDK is supporting clinical research studies that highlight the distinctive position that the Institute, as a public funder of medical research, can hold. Biomedical research is supported by several sources, like industry (pharmaceutical and biotechnology companies), patient advocacy organizations, private foundations, and, in the case of the NIH, the American public. Many successful collaborations have brought different funders together to maximize investments. There are also instances for each of these funders to fill a unique need in promoting research.

The following examples are studies that were designed to answer critical questions in treatment of diseases/disorders that fall under the NIDDK's purview, but were unlikely to have been undertaken by the other funding sources. They are testing generic or off-label (currently approved by the U.S. Food and Drug Administration (FDA) for a specific use or uses, but may be applicable to other uses) medications or behavioral changes and, therefore, would not be profitable to pursue. In some examples, these studies are not testing a particular therapy, but are studies of disease that may inform future therapies. Finally, many of these are large studies that require significant investment that others would simply be unable to fund. While the NIDDK is the lead sponsor of all of these studies, some have benefitted from additional contributions from others, both federal and non-federal, to help advance the research.

Diabetes Prevention and Treatment

Type 2 diabetes is a chronic and costly disease. Therefore, it is critical to determine the best and most affordable ways to prevent and treat the disease. The NIDDK recently started two diabetes trials with potentially high public health impact. Vitamin D has emerged as a potential modifier of type 2 diabetes risk. A large, randomized, and controlled clinical trial, however, is required to prove if vitamin D supplementation prevents or delays type 2 diabetes in adults who are at high risk. Because vitamin D is an inexpensive, generic supplement, it was unlikely that other funders would test its value in diabetes prevention. Therefore, the NIDDK began the Vitamin D for Type 2 diabetes (D2d) Trial, which will enroll over 2,000 participants. D2d could provide the evidence for another affordable and accessible way to help prevent or delay type 2 diabetes.

The diabetes drug metformin and/or lifestyle change are typically used as first-line treatments for type 2 diabetes, but no long-term, head-to-head comparisons have been conducted to determine the best second medication to optimize control of type 2 diabetes, and such studies were unlikely to be undertaken by other funders. The NIDDK's Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE) will compare four commonly used diabetes drugs in combination with metformin. GRADE will compare the drugs for their effects on glucose levels, diabetes complications, and quality of life, along with adverse effects, over an average of nearly five years in 5,000 volunteers. The outcome of the GRADE study will have a major public health impact by establishing the most effective treatments for early therapy of type 2 diabetes, and has the potential to enable clinicians to make personalized medication decisions, based upon the individual characteristics of their patients.

The NIDDK's Look AHEAD (Action for Health in Diabetes) Trial is another trial unlikely to have been conducted by industry as it does not involve medication, but rather tests a behavioral intervention. This clinical trial, launched in 1999, was designed to evaluate the long-term effects—over a decade—of an intensive weight loss intervention in over 5,000 overweight and obese older women and men with type 2 diabetes. Look AHEAD demonstrated

that the intensive lifestyle intervention resulted in sustained and clinically significant weight loss with improvements in cardiovascular risk factors as well as quality of life. Importantly, Look AHEAD determined that the intensive lifestyle intervention does not reduce cardiovascular events, such as heart attack and stroke, but did show other important health benefits such as improved diabetes control and reduced medication use. Look AHEAD continues to follow study participants to determine the long-term health impact of an intensive lifestyle intervention.

Digestive Diseases

In people with the digestive disease gastroparesis, food is slowed or stopped moving from the stomach to small intestine, leading to nausea, a feeling of fullness after eating only a small amount of food, vomiting undigested food, and lack of appetite. Tricyclic antidepressants may block certain pathways which regulate nausea and vomiting, and thus are frequently used for treatment of these symptoms in people with gastroparesis. Yet a large, randomized, and controlled trial was needed to provide evidence as to whether or not this practice benefits people with gastroparesis. Because this is not the disease for which the medication was approved, it was necessary for the clinical trial to be approved by the FDA; these conditions made it less likely that other funders would pursue such a trial. NIDDK's Nortriptyline for Idiopathic Gastroparesis (NORIG) Trial sought to evaluate whether treatment with nortriptyline, a drug used to treat depression, can improve gastroparesis symptoms. The study determined that nortriptyline did not improve overall symptoms in idiopathic gastroparesis over a 15-week period. However, improvements in appetite, satiety, and body mass index (BMI) were noted. NORIG was conducted as part of the Gastroparesis Clinical Research Consortium.

Chronic Kidney Disease

Despite the impact of chronic kidney disease on the nation's health, understanding of its epidemiology—the study of the causes, distribution, and control of disease in populations—remains incomplete. To fill this need, in 2001 the NIDDK established the Chronic Renal Insufficiency Cohort (CRIC) Study to identify risk factors that predict loss of kidney function, as well as the development and worsening of cardiovascular disease, in people with chronic kidney disease. The CRIC Study investigators recruited and are following nearly 3,900 volunteers, the largest and most comprehensive study of chronic kidney disease ever conducted. The CRIC Study investigators characterized this large cohort, contributing numerous important findings to knowledge about chronic kidney disease, and are now conducting extended follow-up of its participants. In addition, the NIDDK also supports a similar study of chronic kidney disease in children, the Chronic Kidney Disease in Children (CKiD) study. The long-term, observational nature of these studies makes them uniquely suited for NIDDK support, allowing investigators to glean new insights into the management and outcomes of chronic kidney disease.

Conclusion

Without NIDDK support, it was unlikely that these important clinical trials and studies would be conducted. In addition to filling critical gaps in knowledge, many of these efforts are collecting extra biosamples that are stored in the NIDDK's Central Repository for further research. This Web-enabled Central Repository makes biological samples and data from NIDDK-funded research available to the broader scientific community. These resources facilitate the testing of hypotheses without the need for new data generation or sample collection. Access to these NIDDK resources is an additional benefit from the public funding of important biomedical clinical trials.

SBIR and STTR Programs: Advancing Health Technology Development Through Public-Private Partnerships

The translation of basic research findings to the development and commercialization of therapeutic drugs, medical devices, and other innovative products remains a fundamental challenge for improving public health. The NIH Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs were designed to address this issue, supporting research and development (R&D) efforts by U.S. small businesses (*i.e.*, companies with no more than 500 employees) to help develop promising and innovative health technologies.

The two programs are similar, in that they include the following objectives: using small businesses to stimulate technological innovation; strengthening the role of small business in meeting public R&D needs; increasing private sector commercialization of innovations developed through public-private partnerships; increasing small business participation in public R&D; and fostering and encouraging participation by socially and economically disadvantaged small businesses, and by and women-owned businesses.

One important difference is that for the SBIR program, the principal investigator's main employment must be with the small business itself; the STTR program has no such requirement, and thus many STTR grantees work primarily at non-profit research institutions (*e.g.*, universities) and cooperatively engage in R&D programs with private sector businesses.

The SBIR/STTR programs are structured in three phases, the first two of which are typically funded by the NIH: Phase I, which aims to establish the technical merit, feasibility, and potential for commercialization of the proposed R&D efforts and to determine the quality of performance of the small business; Phase II, which increases funding of R&D efforts initiated in Phase I, based on scientific and technical merit, as well as commercial potential; and Phase III, which, where appropriate, is for small businesses to pursue, with non-SBIR/STTR funds, the commercialization objectives resulting from the Phase I/II R&D activities. Grantees of the SBIR program are also eligible for technical assistance programs, which offer specialized, strategic business training and provide access to a vast network of industry experts. Support for research that falls within these different phases of the drug and therapy development pipeline is critically important, but has historically been underfunded—resulting in the so-called "valley of death" of investment in the drug and device development process.

Thus, the SBIR/STTR programs are important mechanisms for bridging that valley as a way to translate basic research findings to drugs, devices, and products that can benefit people. The NIDDK funds numerous SBIR/STTR grants supporting a variety of research on promising health technologies relevant to its mission. A few examples among many success stories are highlighted below.

The Sharklet Catheter

Catheter-associated urinary tract infections (CAUTIs) are the leading cause of hospital-acquired infections. CAUTIs are caused by bacteria that colonize the urinary catheter surface, leading to the formation of harmful bacterial sheets, or biofilms, over time. Sharks, unlike other marine animals, do not accumulate bacteria, algae, and other microorganisms on their skin. Shark skin is textured with diamond-shaped features, called denticles, topped with small spines.

This intriguing, naturally occurring design inspired investigators at Sharklet Technologies, Inc. to develop silicone catheters that resist biofilm formation and reduce bacterial colonization. Importantly, the innovative microbial resistance comes from the surface texture, without needing antibiotics, thus reducing the risk for the emergence of drug-resistant bacterial strains. The Sharklet catheter, supported in part by NIDDK through Phase I and II SBIR grants, successfully inhibits

bacterial migration and has now been licensed to a medical device manufacturer.

HemoShear

A great number of drugs fail during the clinical trial process, often due to liver toxicity. Historically, drugs were tested for toxicity using cells grown in the laboratory and in animal models before they were tested in humans in clinical trials. These laboratory and animal systems, however, do not always reflect human liver biology accurately, allowing drugs to continue on to human trials that, after considerable time and expense, could ultimately end in failure because of toxicity.

The company HemoShear, LLC, with NIDDK Phase I and II SBIR support, developed a cell-based system that may more accurately represent the complex biology of the human liver, thereby improving the likelihood of discovering toxicity before costly and time-consuming clinical trials. HemoShear's innovative technology has the potential to save pharmaceutical companies money and time, as well as to spare patients the risk of encountering unexpected toxicity, by allowing companies to focus trials on the most safe and promising candidate drugs. Leading biotechnology and pharmaceutical companies are already working with HemoShear's system to improve their methods for evaluating the safety and efficacy of potential therapeutics.

Artificial Pancreas Technologies

When blood glucose levels are not well controlled, a person with diabetes is at much greater risk for developing devastating health complications, including blindness, kidney disease and kidney failure, nerve problems, and cardiovascular disease. An artificial pancreas, or a "closed-loop system," is technology in which a computer calculates insulin dose based on glucose levels and delivers insulin automatically through an insulin pump with minimal human input. Such technology holds great promise to help people with type 1 diabetes, as well as other populations, safely achieve recommended levels of blood glucose control.

The NIDDK has supported many aspects of research toward the development of an artificial pancreas, to a large extent through SBIR and STTR grants, for at least two decades. For example, all the current continuous glucose monitoring (CGM) technology on the market—technology that measures circulating glucose levels in real time—benefitted from NIDDK support early in development. Those technologies have been approved by the U.S. Food and Drug Administration and are currently being used by patients, but are also an important component of a future artificial pancreas system. In addition to CGM, SBIR/STTR grants support a range of other research critical for artificial pancreas development, including efforts to increase the stabilities of insulin and its opposing hormone glucagon, improve insulin delivery systems, and explore new ways to make artificial pancreas technologies more user-friendly and convenient for people with type 1 diabetes.

Conclusions

These success stories highlight the groundbreaking health technology innovations that can arise from public-private partnerships. New products that result from the SBIR/STTR programs have the potential to improve health, foster exciting new research directions, and reduce the costs of drug development and health care. The SBIR and STTR programs currently are authorized through 2017, and will continue to bridge important gaps in the pipeline of drug and medical device development.

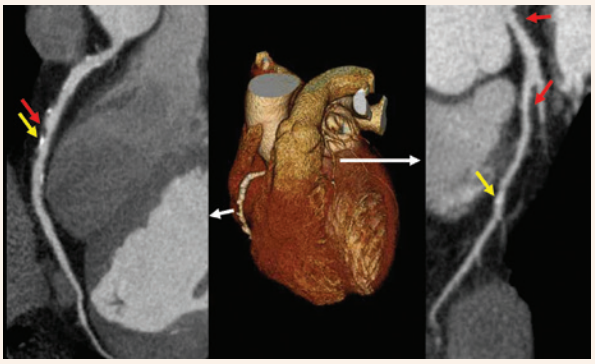
NIDDK-NIBIB's Biomedical and Metabolic Imaging Branch: Collaboration Leads to Better Imaging

In 2011, two NIH Institutes joined forces to share their expertise and sophisticated imaging tools to advance the understanding of cardiovascular disease, diabetes, and other challenging health conditions. The NIDDK and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) have been conducting a joint program called the Biomedical and Metabolic Imaging Branch (BMIB) in the NIH Clinical Research Center.

The BMIB's multidisciplinary team includes imaging physicists, spectroscopists, and clinicians; the daily operations of the branch are managed by NIDDK senior radiologist Dr. Ahmed Gharib. The BMIB's high-quality imaging capabilities provide basic scientists and clinicians with a more accurate depiction of a developing disease, while low-quality images increase the likelihood of misdiagnosis.

For example, a low-resolution liver scan may leave important details—such as tiny tumors—blurry and difficult to identify. High-resolution scans, taken from many angles, however, provide clear and detailed images that enable researchers to pinpoint problems earlier in a disease. Advanced imaging techniques can also be used to examine multiple organs simultaneously, making it possible to understand, detect, and manage various systemic diseases.

Advances in imaging also allow researchers to image parts of the body faster and in greater anatomic and functional detail than ever before. BMIB researchers use a computed-tomography (CT) technique to capture crisp images of the heart's blood vessels while the heart is beating. Imaging a functioning heart is somewhat like photographing a fast-moving car—the more pictures taken, the greater the likelihood that at least one will be clear. Researchers can now capture a full CT scan of the heart in one to two heartbeats, whereas just a decade ago the same scan would have required 16 heartbeats.



BMIB's high-quality imaging capabilities provide basic scientists and clinicians with a more accurate depiction of a developing disease. Here, a three-dimensional reconstructed image of the whole heart (center) is flanked by detailed images of the right coronary artery (right) and left anterior descending coronary artery (left). The red and yellow arrows indicate different types of coronary plaques, which can be early signs of heart disease. ***Photo courtesy of Dr. Ahmed Gharib, from J Clin Endocrinol Metab. 2013 May; 98(5): 2045-2052. Reprinted with permission from Nature Publishing Group.***

The BMIB's use of state-of-the-art imaging equipment and newly developed techniques benefit a variety of patients and support an expansive range of research across NIH. Proton magnetic resonance spectroscopy (MRS), for example, allows researchers to measure not only fat in the liver but also metabolites such as glycogen and choline. Studying these liver metabolites enhances the understanding of lipid and glucose metabolism under the more controlled environment of a metabolic research unit.

The BMIB grew out of NIDDK's Integrated Cardiovascular Imaging Lab, which focused its efforts on demonstrating the link between metabolic syndrome (a combination of factors that increases the risk of heart disease, stroke, and diabetes) and atherogenesis (the formation of lesions in arterial walls). Today, BMIB researchers work with the

NIDDK-led Metabolic Clinical Research Unit (MCRU) to investigate the interrelationship between obesity, coronary heart disease, and other conditions. Obese MCRU patients often go to the BMIB to be scanned in its three-tesla magnetic resonance imaging (MRI) machine that can accommodate people who weigh up to 500 pounds. The imaging techniques used on such patients allow scientists to better understand a range of metabolic diseases.

"The work that we are doing in the branch holds tremendous promise for improved understanding of systemic disease and for early detection methods [especially for] atherosclerosis, which has long been the number one killer in much of the developed world," said NIBIB Director Dr. Roderic Pettigrew. "We hope to see hospitals throughout the world one day using approaches based on this research."

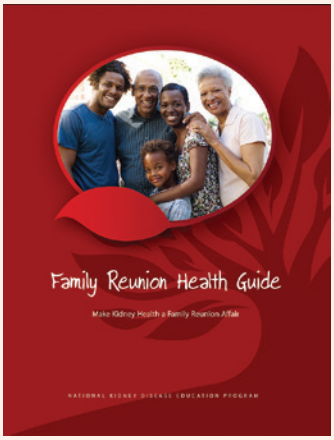
In keeping with NIH's focus on collaboration, BMIB staff scientists Drs. Ronald Ouwerkerk and Khaled Abd-Elmoniem and others work closely with other Institutes—such as the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institute of Allergy and Infectious Diseases, and the National Heart, Lung, and Blood Institute—that also depend on advanced imaging. For example, BMIB investigators have developed MRI and MRS techniques that can assess the metabolic composition of various organs and measure tissue stiffness. High-resolution coronary MRI provides a two-fold improvement over conventional MRI in spatial resolution.

Additionally, improvements in temporal resolution allow researchers to view and measure the thickening of the coronary artery wall, the earliest stage of coronary artery disease. In the future, these new techniques may be used to monitor the effects of therapies and screen people at risk for coronary artery disease as well as metabolic and liver dysfunction.

"The collaborative infrastructure at NIH has enabled all of us to work together more efficiently," said NIDDK Director Dr. Griffin P. Rodgers. "The Biomedical and Metabolic Imaging Branch is an excellent example of what can be accomplished through ongoing partnerships within NIH."

This feature was adapted from an article that originally appeared in the May-June 2013 edition of the NIH Catalyst.

NIDDK Health Information Resources for the Public



The NIDDK's research mission includes some of the most common and consequential conditions affecting Americans today, as well as diseases that are more rare but nonetheless devastating. Every year, people diagnosed with these conditions and their family members ask themselves and their doctors, "What do I need to know? How can I protect my health and my family's health?"

The NIDDK is dedicated to delivering accurate, science-based health information to the public. The NIDDK offers many publications aimed at informing doctors, patients, and families affected by these conditions. A wide variety of informational materials are offered in multiple languages and often in large-print editions. To reach the widest possible audience, the publications are available as free downloads online, and printed materials can be ordered for free or at nominal cost.

All of these resources can be reached through the NIDDK Clearinghouses Publications Catalog at *www.catalog.niddk.nih.gov*

Disease-specific information is available through the:

- National Diabetes Information Clearinghouse
- National Digestive Diseases Information Clearinghouse
- National Kidney and Urologic Diseases Information Clearinghouse
- Weight-control Information Network
- National Diabetes Education Program
- National Kidney Disease Education Program
- National Endocrine and Metabolic Diseases Information Service
- National Hematologic Diseases Information Service

NIDDK Director Dr. Griffin P. Rodgers highlights the dissemination of knowledge through outreach and communication efforts as one of the overarching principles that guide his leadership and vision for the NIDDK. As Dr. Rodgers states, "We are continuing efforts to ensure that the science-based knowledge gained from NIDDK-funded research is imparted to health care providers and the public for the direct benefit of patients and their families."

This poster announced the "Imaging the Pancreatic Beta Cell Workshop" held in April 2013, which was co-sponsored by the NIDDK, JDRF, and the European Union. Shown on the poster is a human pancreatic islet, which contains many different cell types, including insulin-producing beta (β) cells (stained green) and glucagon producing alpha cells (stained red). In type 1 and type 2 diabetes, β cells are lost or cease secreting enough insulin to regulate a person's blood glucose levels. At the workshop, scientists discussed recent research progress and emerging opportunities in β cell imaging. The objective of this research is to develop imaging approaches that could be used to monitor the mass, function, and inflammation of naturally occurring or transplanted β cells in the body, in people with type 1 or type 2 diabetes, or in people who are at risk for these diseases. Imaging the β cell holds promise as a means to allow scientists to visualize the extent of pancreatic damage and, potentially, to see directly if a therapy is effective.

Islet image provided by the laboratory of Dr. Alvin Powers, Vanderbilt University.

Diabetes, Endocrinology, and Metabolic Diseases

NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, these diseases and conditions affect many millions of Americans and can profoundly decrease quality of life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.

Diabetes is a debilitating disease that affects an estimated 25.8 million people in the United States—or 8.3 percent of the total population—and is the seventh leading cause of death.[1] Compared with people of similar age without the disease, diabetes doubles people's overall risk of death, as well as increases their risk of death from cardiovascular disease two- to four-fold. Diabetes is also the leading cause of kidney failure, nontraumatic lower limb amputations, and, in working-age adults, blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2012—including costs of medical care, disability, and premature death—was $245 billion.[2] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin, and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production. In addition, a significant proportion of pregnant women each year are diagnosed with gestational diabetes, a form of diabetes that is similar to type 2 diabetes but unique to pregnancy. Untreated, any form of diabetes during pregnancy increases the risk of serious complications for the mother and baby before, during, and after delivery.

Type 1 diabetes, formerly known as juvenile diabetes, affects approximately five percent of adults and the majority of children and youth with diagnosed diabetes.[1] It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing beta (β) cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that keeping blood glucose levels as near to normal as safely possible reduced the risk of eye, kidney, nerve, and heart complications associated

[1] *2011 National Diabetes Fact Sheet. Centers for Disease Control and Prevention. Atlanta, GA.*

[2] *American Diabetes Association. Diabetes Care 36: 1033-1046, 2013.*

with type 1 diabetes. Thirty years after the launch of the DCCT in 1983, the DCCT and EDIC studies continue to provide important information about type 1 diabetes and its complications. However, despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to levels achieved by functional β cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, including technology to link them in an "artificial pancreas," as well as working to develop β cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.[1] Type 2 diabetes is associated with several factors, including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at higher rates among minority groups, including African Americans, Hispanic and Latino Americans, American Indians, and Native Hawaiians and Pacific Islanders.[1] Gestational diabetes is also a risk factor: women who have had gestational diabetes have a 35 to 60 percent chance of developing diabetes—mostly type 2 diabetes—in the next 10 to 20 years.[1]

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the pancreatic β cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. Treatment approaches for controlling glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 79 million adults in the United States who have a condition called "prediabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[1] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a seven percent reduction in body weight. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 years.

Type 2 diabetes was previously called "adult-onset" diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. For example, the NIDDK-supported Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial showed that the disease may be more aggressive and difficult to treat in youth compared to adults. This is worrisome because the onset and severity of disease complications correlate with both the duration of diabetes and control of blood glucose levels; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. The TODAY study continues to provide valuable information on type 2 diabetes in youth, including data on poor risk factor control and emerging complications among study participants, highlighting the urgent need for further research toward effectively preventing and treating type 2 diabetes in youth. In addition, increasing rates of type 2 diabetes in girls may lead to more women who enter pregnancy with diabetes, and maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of type 2 diabetes in offspring. Thus, the rising rates of diabetes and prediabetes in young women could lead to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

The NIDDK is supporting research to better understand metabolism and the mechanisms that lead to the

[3] *Eberhardt MS, et al. MMWR 53: 1066-1068, 2004.*

development and progression of diabetes and the many other endocrine and metabolic diseases within the NIDDK's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

BETA CELLS AND DIABETES

New Class of Genes Involved in Beta Cell Maturation and Diabetes: Researchers have identified a new class of genes that plays a role in maturation of pancreatic insulin-producing beta (β) cells and may be involved in diabetes. RNA has been known historically as the intermediate between DNA and proteins: DNA is decoded into RNA, which in turn is translated into protein. However, recent research has discovered the presence of numerous RNA molecules that are encoded from DNA but not translated into proteins. A subset of these RNAs is called "long non-coding RNAs" (lncRNAs), and their function is largely unknown, although research suggests that some may be involved in regulating whether genes are turned on or off (gene expression). While many protein-coding RNAs are often present in several different cell types, lncRNAs are often found only in a single cell type. This observation suggests that lncRNAs may regulate cell-specific tasks, and potentially makes them attractive targets for therapy to affect one cell type.

Because β cells are central to the development of both type 1 and type 2 diabetes, researchers sought to identify lncRNAs that were expressed in human pancreatic islets, which are primarily composed of β cells. They identified over 1,100 lncRNAs expressed in islets, with about half of them expressed in islets only. They next looked at a subset of lncRNAs, to determine when they were expressed during β cell development. The lncRNAs were not present in early progenitor cells, but were active in mature islets, suggesting that they may play a role in promoting β cell maturation. Additional experiments suggested that lncRNAs may function by controlling the expression of islet-specific genes. These findings are important because a major goal of diabetes research is to identify strategies to turn progenitor cells into mature β cells, and this research has identified a class of molecules that may be involved in this process and/or could be markers of mature β cells.

The researchers next examined whether the lncRNAs may play a role in type 2 diabetes. They discovered a small number of lncRNAs that were abnormally expressed in human islets from people with type 2 diabetes, and others that were located near genetic regions previously shown to be associated with susceptibility to type 2 diabetes, suggesting that lncRNA abnormalities may explain part of the genetic susceptibility to type 2 diabetes. This research has therefore identified a new class of genes that are expressed in islets, promote maturation of β cells, and may play a role in type 2 diabetes. It also opens up new avenues for studying and potentially promoting β cell maturation, and for examining the underlying genetic causes of diabetes toward identifying new targets for therapy.

Morán I, Akerman I, van de Bunt M, et al. Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. Cell Metab 16: 435-448, 2012.

Protein on Surface of Beta Cells Is Possible Type 2 Diabetes Drug Target: Researchers discovered that a protein found on the surface of insulin-producing beta (β) cells in the pancreas may be an attractive drug target to prevent or treat type 2 diabetes. In type 2 diabetes, β cells do not release enough insulin to maintain healthy blood glucose levels. This defect is due to impairments in β cell function combined with reduced numbers of β cells (referred to as β cell mass). Scientists examined whether turning on (activating) a protein—called a G_q-coupled receptor—found on the surface of β cells would have an effect on β cell mass or function in mice. A related protein, a G_s-coupled receptor, is already the target of type 2 diabetes drugs in humans, so the researchers wanted to determine if the G_q-coupled receptor could also be a potential drug target. Like the G_s form of the protein, the G_q form is found on

many different cell types. Thus, to study the G_q form in β cells only, they used an experimental mouse model they had previously generated, which made a "designer" G_q-coupled receptor specifically in β cells. The designer receptor could be activated by giving the animals a compound that was otherwise biologically inert.

The scientists discovered that chronically activating the designer G_q-coupled receptor with the compound resulted in a robust increase in the animals' β cell mass and function. This was associated with increased expression (turning on) of several genes known to be involved in promoting β cell development or maintaining normal β cell function. Activating the receptor prevented the animals from developing diabetes induced by a toxin that destroys a large percentage of β cells. It also prevented the metabolic defects seen when mice eat a high-fat diet. These results suggest that G_q-coupled receptors play an important role in regulating β cell function and blood glucose levels in mice. If they play a similar role in humans, they would be attractive targets for drug development to combat type 2 diabetes. Because the receptors are found on other cell types, therapeutic approaches would need to activate receptors found on β cells specifically so as to minimize side effects.

Jain S, Ruiz de Azua I, Lu H, White MF, Guettier JM, and Wess J. Chronic activation of a designer G_q-coupled receptor improves β cell function. J Clin Invest 123: 1750-1762, 2013.

Newly Discovered Hormone Increases Beta Cell Proliferation in Mice: Researchers have discovered a new hormone, called betatrophin, that promotes pancreatic beta (β) cell proliferation and improves glucose control in mice. Scientists hope the finding may lead to new ways to prevent or slow the progression of diabetes in humans. β cells, which produce the hormone insulin, are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace lost β cells and restore the body's insulin-producing capacity would benefit people with type 1 or type 2 diabetes and is a major research goal. Previous research found that blocking insulin signaling in tissues such as the liver resulted in β cell proliferation and increased insulin secretion. To determine what causes this effect, and to provide clues to what cellular factors may regulate β cell proliferation, researchers treated mice with a molecule that blocked insulin signaling and then studied what genes were turned on as β cells proliferated. They identified a gene that was turned on in the liver and fat of treated mice. The gene was found to encode a protein, which they named betatrophin. The researchers discovered that the protein is secreted by the liver and fat, and travels through the bloodstream. Increasing the amount of betatrophin produced in mice tripled the mass of their pancreatic β cells in just eight days, doubled insulin production, and led to a lower fasting glucose level and improved glucose tolerance compared to control mice. Humans have a very similar gene, though whether or not human betatrophin plays a similar role as mouse betatrophin is currently under investigation. Research to understand what proteins betatrophin interacts with, how betatrophin stimulates β cell proliferation, and what betatrophin's other functions might be is still ongoing and could possibly lead to new therapies.

Yi P, Park JS, and Melton DA. Betatrophin: a hormone that controls pancreatic β cell proliferation. Cell 153: 747-758, 2013.

ADVANCING TECHNOLOGY IN DIABETES MANAGEMENT

Smartphone Technology Advances Progress Toward the Development of an Artificial Pancreas for People with Type 1 Diabetes: Researchers used smartphone technology to move a step closer toward developing an artificial pancreas. An artificial pancreas, or a "closed-loop system," is technology in which a computer calculates insulin dose based on glucose levels and delivers insulin automatically through an insulin pump with minimal human input. Such technology holds great promise to help people safely achieve recommended levels of blood glucose control, as well as to alleviate an enormous amount of patient burden associated with current self-management strategies. Significant progress toward developing an artificial pancreas has been achieved in recent years, with researchers testing closed-loop systems in people in hospital settings using

laptop computers, which limits mobility. To realize the promise of this technology, patients need a portable and wearable system so that they can carry out activities of daily life.

In new research, scientists tested whether smart phone technology could replace laptops to control a closed-loop system. They tested their new technology, called the Diabetes Assistant (DiAs), in 20 people with type 1 diabetes at four sites in the United States and Europe. DiAs (on a smartphone) communicated wirelessly with a communication box, which in turn signaled wirelessly to a continuous glucose monitor (which measures blood glucose levels) and an insulin pump. After participants were given a brief orientation about how to use DiAs, they used the system on their own. Participants stayed in real world settings, such as hotels, and ate whatever they wanted; to ensure that they were safe at all times, they were closely monitored by study personnel.

The study found that the artificial pancreas system had proper system communication 98 percent of the time, which was higher than the goal of 80 percent in this study. The researchers concluded that smartphone technology could be used to run a closed-loop system, and that DiAs is a promising platform for future study. The technology has already advanced since this study was conducted, and the intermediate device (communication box) is being phased out. This research is an important step forward toward developing a portable, usable, and safe artificial pancreas system, and sets the stage for future clinical trials.

Kovatchev BP, Renard E, Cobelli C, et al. Feasibility of outpatient fully integrated closed-loop control: first studies of wearable artificial pancreas. Diabetes Care 36: 1851-1858, 2013.

CARDIOVASCULAR DISEASE RISK IN YOUTH WITH TYPE 1 DIABETES

Increased Understanding of Cardiovascular Disease Risk Factors in Youth with Type 1 Diabetes: Two studies of cardiovascular disease (CVD) risk factors in youth with type 1 diabetes provide new information about progression of CVD in this population, and about potential prevention strategies. Type 1 diabetes, like type 2 diabetes, is associated with an array of serious, long-term complications, including CVD. People with diabetes have a greatly increased risk for CVD, CVD occurs earlier in people with diabetes than in people without diabetes, and CVD is the leading cause of mortality in people with diabetes. Youth with type 1 diabetes will endure a higher lifetime burden of diabetes than adults and, therefore, a higher burden of complications, including CVD. It is not known, however, whether CVD in youth with type 1 diabetes has similar risk factors and/or the same progression to advanced disease as in adults. By studying the natural history of CVD and examining CVD risk factors in youth with type 1 diabetes, scientists from the SEARCH for Diabetes in Youth CVD Study gained insights that could lead to strategies to prevent or treat CVD in this population.

In the first study, SEARCH scientists examined heart rate variability in youth with type 1 diabetes. The autonomic nervous system regulates heart rate, enabling changes in heart rate in response to activity/stress and rest. Reduced heart rate variability is a sign of cardiac autonomic neuropathy, a complication of diabetes that increases the risk of mortality. The researchers found that youth with type 1 diabetes have reduced overall heart rate variability, particularly youth with suboptimal blood glucose control, compared to youth without type 1 diabetes. In the other study, SEARCH scientists examined the "carotid intima-media thickness" (IMT) of youth with type 1 diabetes. IMT is a measure of the thickness of the arterial wall of the carotid artery, which supplies the head and neck with oxygenated blood. A thickening of the arterial walls can indicate the presence of atherosclerosis. In adults, type 1 diabetes leads to an increase in carotid IMT. In this study, the researchers found that youth with the disease also had increased IMT and that this association may be attributable to poor blood glucose control, compared to youth without type 1 diabetes. Therefore, both heart rate variability and IMT are altered in youth with type 1 diabetes, indicating that this population shows signs of CVD risk early in the course of the disease.

The NIDDK's Diabetes Control and Complications Trial (DCCT) and its follow-up study, Epidemiology of Diabetes Interventions and Complications, found that adults with type 1 diabetes who had intensive blood glucose control during the DCCT had about half the rate of CVD events compared to those who received conventional treatment. The SEARCH CVD study has provided novel data about CVD and its risk factors in youth with type 1 diabetes, and the findings continue to suggest the importance of good blood glucose control to prevent CVD in people with the disease.

Jaiswal M, Urbina EM, Wadwa RP, et al. Reduced heart rate variability among youth with type 1 diabetes: the SEARCH CVD study. Diabetes Care 36: 157-162, 2013.

Urbina EM, Dabelea D, D'Agostino RB Jr, et al. Effect of type 1 diabetes on carotid structure and function in adolescents and young adults: the SEARCH CVD study. Diabetes Care 36: 2597-2599, 2013.

UNDERSTANDING THE MECHANISM OF A DRUG USED TO TREAT TYPE 2 DIABETES

New Mechanism Proposed for Glucose-lowering Effect of Metformin, a Drug for Type 2 Diabetes: A new report may explain how the diabetes drug metformin exerts its effects, providing a potential target for development of new therapeutics. The liver plays an important role in blood glucose (sugar) levels by sending glucose into the bloodstream when levels start to drop between meals. Liver glucose output during fasting is triggered by glucagon, a hormone secreted by the pancreas that works in opposition to another hormone, insulin, which the pancreas secretes when blood glucose levels are high (after meals). In people with type 2 diabetes, not only does the body become resistant to the glucose-lowering effect of insulin, but it also produces glucagon inappropriately. As a result, glucose enters the bloodstream from the liver when it is not needed, contributing to high blood glucose levels. The most commonly prescribed oral drug for treatment of type 2 diabetes, metformin, helps control blood glucose levels by reducing the amount of glucose coming from the liver. How it is able to do this has been unclear. Metformin treatment causes an increase in the levels of a molecule called AMP. AMP activates an enzyme called AMPK, and the activated AMPK was thought to reduce liver glucose production, accounting for metformin's effects. Now, working in mouse liver cells and mouse models, researchers have uncovered evidence strongly suggesting that metformin interferes with glucagon-induced glucose output by disrupting the initial steps of glucagon signaling in liver cells. In particular, their data suggest that the metformin-induced rise in AMP inhibits glucagon's activation of an enzyme called adenylate cyclase, the first step in the molecular signal for glucose production inside liver cells. If this theory is borne out, it paves the way to developing potential new drug treatments for type 2 diabetes that specifically target this enzyme. These findings also reinforce the importance of glucagon and finding ways to minimize its abnormal activity in type 2 diabetes.

Miller RA, Chu Q, Xie J, Foretz M, Viollet B, and Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494: 256-260, 2013.

ADVANCES IN CYSTIC FIBROSIS TESTING AND TREATMENT

An Important Proof of Principle for the "Combination Therapy" Approach to Treating the Most Common Cystic Fibrosis Mutation: New research has shown that it may one day be possible to treat people with cystic fibrosis (CF) using a combination of medicines that work cooperatively to stabilize an aberrant form of CFTR, the protein that is defective in CF. Among people with CF, the most common genetic mutation causing the disease is designated *cftr-ΔF508*. The U.S. Food and Drug Administration recently approved a "small molecule corrector" drug that alleviates CF when it is caused by a less common *CFTR* mutation (found in roughly five percent of CF patients), allowing patients with the rarer mutation to lead their lives with far fewer symptoms of the disease. However, researchers have not yet been successful in taking a similar approach

to find small molecule correctors for the *cftr-ΔF508* mutation. While several candidate small molecule drugs have been found to improve stability of the cftr-ΔF508 protein in the laboratory, clinical trials are still under way with people with this mutation, so their therapeutic value is not yet known. Previous NIDDK-supported research identified a possible explanation: *ΔF508* destabilizes multiple "domains" of the protein (distinct sections of the protein which fulfill specific biological roles). In particular, *ΔF508* disrupts two "nucleotide binding domains" (designated NBD1 and NBD2), as well as the interaction of NBD1 with critical regions that connect parts of the protein that are inside the cell with a portion that is outside the cell, termed "membrane spanning" domains. The new study characterizes the existing candidate drugs in terms of which domain they stabilize, effectively defining three classes of corrector compounds. Existing correctors stabilize the interaction of NBD1 with the first and second membrane spanning domains (class I) or stabilize NBD2 (class II). Also necessary is stabilization of NBD1 itself (class III), achieved in this study by using the chemical glycerol or other "chemical chaperones." In a key test on human lung cells grown in the laboratory that harbored the *cftr-ΔF508* mutation, they found that using only one or two classes of correctors resulted in very little CFTR activity; but combined treatment with all three classes of correctors achieved almost normal levels of CFTR function. The chemical chaperones that serve as class III correctors in this study are unlikely themselves to be medicinally useful. For example, because glycerol is a naturally occurring compound that is freely metabolized by the body, it would not reach target tissues in sufficient concentration to function as a therapeutic agent. However, these results strongly suggest a potential benefit for focusing efforts on finding compounds that stabilize the NBD1 domain of the cftr-ΔF508 protein. The research suggests that if a compound that can do so proves safe in combination with existing class I and class II correctors, the approach may greatly improve treatment for the majority of people with CF.

Okiyoneda T, Veit G, Dekkers JF, et al. Mechanism-based corrector combination restores ΔF508-CFTR folding and function. Nat Chem Biol 9: 444-454, 2013.

Improving Genetic Testing for Cystic Fibrosis:
New research has greatly expanded knowledge of the specific mutations capable of causing cystic fibrosis (CF). Previous landmark research established that CF is caused by mutations in a gene, *CFTR*, encoding a critical channel protein that enables the movement of chloride in and out of cells. Everyone has two copies of *CFTR*, one inherited from each parent. Those with CF have mutations that disrupt the function of both of their *CFTR* copies. People with a single CF-causing mutation are often unaware of it, because their one functioning *CFTR* copy is enough to keep them healthy. Therefore, when considering having children, couples with CF in one of their families will often pursue genetic testing and counseling to determine the likelihood that one of their children will have the disease. If one copy of a known CF-causing mutation is found through either prenatal or newborn screening, it may be uncertain whether a variant in the baby's other copy of *CFTR* would lead to the development of CF. However, although research has identified over 2,000 variations in the *CFTR* gene, only 23 of these were previously shown to be capable of causing CF. While most of the other known variants are likely to be harmless, sometimes genetic testing reveals a variant of unknown significance in potential parents, so genetic counselors cannot tell them with confidence what their odds are of conceiving a child with CF.

To better understand which *CFTR* mutations pose a risk of causing CF, researchers examined genetic records of almost 40,000 people diagnosed with CF—estimated to be more than half of the world population with the disease—for whom clinical measurements of CFTR chloride channel function had been recorded. Confining their analysis to 159 variants found in at least 0.01 percent (one in 10,000) of people with CF in the database they had assembled, the researchers tested how these *CFTR* mutations affect chloride transport when introduced into cells grown in the laboratory, and compared the results of those tests to clinical measurements of CF from their database. As a result, they were able to unambiguously identify 104 specific CF-causing *CFTR* variants in addition to those already known, for a total of 127. For the remaining 32 variants, the clinical data appeared

inconsistent with CF, or the laboratory function tests indicated a variant that should function reasonably well, or both. This may at first seem surprising, since a person with CF should not have a fully functional copy of *CFTR*, but in some cases a benign change may lie alongside a second *CFTR* mutation that does cause disease. Other variants may ordinarily be harmless, but might have the capacity to promote CF symptoms in people with other (unknown) genetic or environmental risk factors. Because men carrying a CF-causing mutation must also have one normally functioning copy of the *CFTR* gene in order to be fertile, the researchers furthered their analysis by examining fathers of CF patients to see if any of them had one of the remaining 32 variants in one copy of their *CFTR* genes, along with a known disease-causing variant in the other. Ten of the 32 variants were found to occur more frequently among the healthy fathers than among people with CF, indicating they do not normally cause disease, even when the other *CFTR* copy contains a disease-causing mutation. Two more were ruled out as disease-causing for other reasons. The remaining 20 variants were not more common among the healthy fathers and could not be ruled out in other ways, so it remains possible that they contribute to disease. As a result of these findings, CF genetic testing will be much more comprehensive, and provide greater certainty to parents concerned about their children's chance of having the disease. Moreover, the laboratory models of the different *CFTR* variants created through this research may promote better understanding of the physiology of the CFTR chloride channel, and may one day lead to improvements in CF patient care.

Sosnay PR, Siklosi KR, Van Goor F, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 45: 1160-1167, 2013.

INSIGHTS ON RARE METABOLIC DISORDERS

Discoveries May Improve Treatment of Mucopolysaccharidosis: Two recent studies in animal models have reported encouraging findings in the quest to improve treatment for people with mucopolysaccharidosis. In this group of genetic diseases, the lack of any one of several enzymes used by cells throughout the body to digest and recycle substances they no longer need leads to accumulation of one or more cellular waste products. These undigested substances are stored in the cells, where they cause serious problems that can include weakness, severe pain, brittle bones, intellectual disability, clouding of the cornea of the eye, organ failure, and death. Periodic infusions of purified replacement enzymes, which cells can absorb and utilize, have been developed as therapies for three types of mucopolysaccharidosis: type I, type II, and type VI (MPS I, MPS II, and MPS VI).

While enzyme replacement greatly alleviates many disease symptoms, lengthening and improving quality of life, such therapies are not cures. A major limitation of the approach has been that the replacement enzymes do not reach some of the tissues where they are needed, including bone, cartilage, and brain, which is separated from the blood by the so-called blood-brain barrier. Therefore, the enzyme therapies provide little or no relief from cognitive and other neurological disease manifestations.

Researchers recently identified a potential solution to this problem, and reported encouraging results in experiments with mice lacking the same enzyme as people with MPS I. The scientists noted that the blood-brain barrier is crossed by a small number of other proteins, and identified the portion of one of these proteins that allows it to move from blood into the brain. They then attached the MPS I replacement enzyme to that key part of the blood-brain barrier-crossing protein and showed that the resulting hybrid protein reached brain cells of MPS I mice, where it dramatically reduced levels of accumulated cellular waste.

Research from a different group has identified another potential therapeutic improvement. Researchers have found that bone and cartilage problems in animal models of various mucopolysaccharidoses are similar to those in people with these diseases. They also found that these problems result, at least in part,

from inflammation triggered by accumulated cellular waste products. These observations suggest that an anti-inflammatory medication might lessen some symptoms in bone and other tissues inaccessible to replacement enzymes or when enzyme replacement therapy is not available. The researchers, therefore, tested pentosan polysulfate, a medication known to reduce inflammation and promote cartilage growth that is approved by the U.S. Food and Drug Administration for treatment of a painful urologic condition, interstitial cystitis/painful bladder syndrome. When the drug was given to rats with MPS VI, the researchers observed significant improvements in bone, cartilage, and tooth structure, as well as some other symptoms. The treated rats were also more mobile and behaved more normally than did untreated control animals, even though cellular waste products continued to accumulate in their bodies.

Further research will be necessary to determine if modifying replacement enzymes to allow their transport across the blood-brain barrier or treatment with pentosan polysulfate—alone or in conjunction with other therapies—may be safe and therapeutically beneficial treatment approaches for lysosomal storage disorders. If so, they may one day allow people with these diseases to lead longer, healthier lives.

Wang D, El-Amouri SS, Dai M, et al. Engineering a lysosomal enzyme with a derivative of receptor-binding domain of apoE enables delivery across the blood-brain barrier. Proc Natl Acad Sci USA 110: 2999-3004, 2013.

Schuchman EH, Ge Y, Lai A, et al. Pentosan polysulfate: a novel therapy for the mucopolysaccharidoses. PLoS One 8: e54459, 2013.

The Course of Type 2 Diabetes and Complication Onset in Youth Today

Four new analyses of data from the Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) trial have revealed important information about the challenges of controlling type 2 diabetes progression and preventing its complications in young people. Although type 2 diabetes is most commonly diagnosed in people over the age of 40, an increase in childhood obesity and other factors has led to a significant rise in cases in people under 20 years of age. Prior to this study, it was unknown whether treatments developed for adults would work well for younger patients. TODAY tested how well three treatment approaches controlled blood glucose levels in ethnically and racially diverse youth ages 10 to 17 who were overweight or obese and had been diagnosed with type 2 diabetes no more than two years before enrollment in the study. All participants received metformin, the first-line drug of choice among adults with type 2 diabetes, and currently the only oral medication approved for use in children. Participants were randomly assigned to receive metformin alone; metformin plus another diabetes drug, rosiglitazone; or metformin plus a program of intensive lifestyle changes aimed at helping participants lose weight and increase physical activity. Unfortunately, metformin alone failed to maintain acceptable, long-term, blood glucose control in most participants over an average follow-up of 46 months—a much higher failure rate than expected—and the addition of the lifestyle intervention provided only a modest improvement that was not statistically significant. Although blood glucose levels remained healthier, on average, in participants who received both metformin and rosiglitazone than in the other groups, the two-drug combination still failed 38.6 percent of the time over the course of the study. Importantly, after the trial began, the U.S. Food and Drug Administration (FDA) restricted use of rosiglitazone because of studies linking the medicine to a higher risk of heart attacks and stroke in adults. Although the FDA recently lifted restrictions on use of rosiglitazone in adults, the drug remains unapproved for use in children.

Since publishing their initial results in 2012, TODAY researchers have examined the data to glean as much as possible about the way the different study treatments affected the participants' metabolic function, as well as their progression toward some of the serious complications of the disease. The cells of people developing type 2 diabetes do not absorb as much glucose as they should in response to insulin. At first, the pancreas compensates by producing more insulin, but when the organ can no longer keep up with demand, diabetes results. In most adults with type 2 diabetes, the pancreas gradually loses the capacity to produce insulin as the disease progresses, so supplementary insulin often becomes required. To better understand the way the disease progressed in youth in the three TODAY treatment groups, study scientists analyzed changes in participants' insulin resistance and capacity for pancreatic insulin production over the course of the trial. They found that average insulin sensitivity gradually fell for the metformin and metformin plus lifestyle groups, while for the metformin plus rosiglitazone group it improved significantly in the first six months of the trial, but later gradually fell back to initial levels.

Thus, at the end of the trial, average insulin sensitivity among those youth getting both medicines was about where it began, but it had worsened among those getting just metformin or metformin plus lifestyle. In contrast, insulin-production capacity fell similarly in all three groups. Importantly, TODAY scientists found that in all of the treatment groups, the participants with the poorest blood glucose control and insulin production at the beginning of the trial were the ones most likely to have higher than recommended blood glucose levels before the study ended. This points to the importance of beginning treatment for pediatric type 2 diabetes before significant loss of insulin production capacity or deterioration of blood glucose control occurs.

One of the major concerns about the increasing frequency with which type 2 diabetes is appearing in

young people is that data from studies in adults show that the onset and severity of diabetes complications correlate with the duration of the disease. Thus, there is great concern that having diabetes for the vast majority of their lives may leave these young people vulnerable to particularly early and severe disease complications.

TODAY researchers, therefore, examined participants for early signs of retinopathy, a diabetes complication of the eye that is the most common cause of adult-onset blindness. Study scientists photographed a majority of participants' retinas during the last year of the study and found that retinopathy was beginning to develop in almost 14 percent of those participants. Among the TODAY participants, increasing time since initial diagnosis of diabetes, greater age, and poorer blood glucose control were all associated with a greater likelihood of retinopathy, reinforcing the importance of early treatment of type 2 diabetes. At multiple stages during the trial, TODAY participants were also tested for key complication risk factors and early signs of kidney and heart complications. Thus, for example, TODAY researchers identified hypertension (high blood pressure)—a major risk factor for both heart attack and kidney disease, both common complications of diabetes—in more than 11 percent of participants when the study began, and almost 34 percent by the end of the study, an average of less than five years after developing diabetes. Boys were more likely than girls to have hypertension or develop it over the course of the trial. The more obese participants were, the more likely they were to have or develop high blood pressure, as well. These findings parallel results in adolescents who do not have diabetes, among whom obesity and male sex are risk factors for developing hypertension.

The researchers also looked for an early sign of kidney disease, the presence of a protein called albumin in urine. More than six percent of TODAY participants had elevated urine albumin when the study began, and more than 16 percent did when it ended; elevated urine albumin was most likely to occur in participants whose blood glucose was more poorly controlled. Two major risk factors for heart disease, dyslipidemia (the elevation of unhealthy blood fats and/or lowering of healthy blood fat levels) and signs of chronic inflammation, were also found to be present at concerning levels when the study began, and at increasing rates as it progressed. In general, none of the treatment approaches were clearly superior to the others at preventing the development of diabetes complications or their risk factors in the TODAY participants, underscoring the importance of further research to identify better ways to prevent, treat, or cure type 2 diabetes in the young.

The TODAY Study Group. Effects of metformin, metformin plus rosiglitazone, and metformin plus lifestyle on insulin sensitivity and β-cell function in TODAY. Diabetes Care 36: 1749-1757, 2013.

The TODAY Study Group. Retinopathy in youth with type 2 diabetes participating in the TODAY clinical trial. Diabetes Care 36: 1772-1774, 2013.

The TODAY Study Group. Rapid rise in hypertension and nephropathy in youth with type 2 diabetes: the TODAY clinical trial. Diabetes Care 36: 1735-1741, 2013.

The TODAY Study Group. Lipid and inflammatory cardiovascular risk worsens over 3 years in youth with type 2 diabetes: the TODAY clinical trial. Diabetes Care 36: 1758-1764, 2013.

Collaborative Efforts Key to Catalyzing Creation of an Artificial Pancreas

On April 9 and 10, 2013, dozens of scientists, engineers, and clinicians from North America, Europe, and the Middle East gathered on the NIH campus with the goal of improving the health and quality of life of people with diabetes. They were participants in the "Workshop on Innovation Towards an Artificial Pancreas," a forum on research and opportunities to accelerate the development and delivery of a promising technological approach to treatment and management of diabetes: a wearable, automated artificial pancreas. Organized by the NIDDK, the lead NIH Institute for artificial pancreas research; the U.S. Food and Drug Administration (FDA), the federal agency regulating medical devices; and the leading health advocacy group for people with type 1 diabetes, JDRF, this workshop represented just one of the latest in a continuing series of collaborative efforts that are helping to make the artificial pancreas a reality.

Why an Artificial Pancreas?

Both type 1 and type 2 diabetes cause the levels of glucose, or sugar, in the blood to rise above a normal, healthy range. When blood glucose levels are not well controlled, a person with diabetes is at much greater risk for developing devastating health complications, including blindness, kidney disease and kidney failure, nerve problems, and cardiovascular disease. These complications exact a heavy toll on both individuals and the health care system in terms of health and health care costs. Thus, finding ways to help people with diabetes optimize control of their blood glucose levels is a critical goal for research on diabetes treatment and management.

For people with type 1 diabetes, controlling blood glucose levels is especially challenging. Type 1 diabetes destroys the insulin-producing beta (β) cells of the pancreas. Without β cells, the pancreas is unable to sense rising glucose levels and respond with secretion of insulin, the hormone that enables the body to absorb glucose—the main source of energy derived from digestion of food—from the bloodstream. To try and keep blood glucose levels within a healthy range, people with type 1 diabetes must measure glucose levels multiple times daily and, based on complex calculations, administer insulin via injection or a pump. While recent advances in technology such as continuous glucose monitors (CGMs) and "smart" insulin pumps that can help calculate insulin doses have helped many patients, recapitulating the dynamic control of blood glucose levels imposed by the β cells of the pancreas is still impossible with current methods. Also, whereas a healthy pancreas has biological safeguards to help prevent blood glucose from dropping too low as well as rising too high, insulin therapy brings with it the risk of potentially life-threatening episodes of low blood glucose, or hypoglycemia, especially at night. Thus, researchers have been working intensively to advance technology that can replace the exquisite control of blood glucose by the pancreas, hoping to achieve an "artificial pancreas" that automatically closes the loop between glucose sensing and delivery of appropriate amounts of insulin, while also preventing hypoglycemia and imposing minimal burden on the user.

Essential components of an artificial pancreas are:

- A glucose-sensing component (sensor) that measures blood glucose levels and sends information to a computer;
- An insulin delivery device, such as an insulin pump; and
- A computer that calculates the amount of insulin needed and thereby "closes the loop" between glucose sensing and insulin delivery.

Working Together To Advance the Field

The design of any system capable of achieving what is expected of an artificial pancreas is complex, and raises novel scientific, clinical, and regulatory challenges. Thus, the NIDDK is working with the FDA and JDRF to help overcome these challenges so that safe and effective artificial pancreas systems can be developed and moved swiftly to market. For example, following a joint workshop on the artificial pancreas organized by the NIDDK, the FDA, and JDRF in 2005, the FDA in 2006 identified "Accelerating the Availability of the Artificial Pancreas" as one of its "critical path" initiatives—FDA's strategy to drive innovation in the scientific processes through which medical products are developed, evaluated, and manufactured. Then, in 2007, the FDA partnered with the NIDDK and other NIH Institutes to develop a working group of federal scientists, clinicians, and regulatory experts who would work together and with stakeholders, such as JDRF, academic researchers, and industry, to find ways to accelerate and optimize research and development efforts toward an artificial pancreas. This Interagency Artificial Pancreas Working Group meets regularly to discuss research and regulatory issues surrounding this new technology, and has been instrumental in promoting the field, including contributing to the development and release of the FDA comprehensive guidance for artificial pancreas systems in November 2012. This guidance is helping researchers in academia and industry identify steps they need to take in the development of their artificial pancreas systems before applying to the FDA for permission to test device safety and effectiveness in people or to market their systems. Also, the April 2013 artificial pancreas workshop was the fourth such collaborative workshop organized by the NIDDK, the FDA, and JDRF in less than a decade to stimulate discussion of the current state of the art in artificial pancreas technology, technical difficulties and possible solutions, safety issues, and next steps.

While the NIH has supported research in this field for over two decades, in the last several years the NIDDK has intensified its artificial pancreas research program as well as its collaborations with other NIH Institutes and JDRF. For example, since 2008, the NIDDK, in partnership with the National Institute of Biomedical Imaging and Bioengineering (NIBIB) and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), has released four funding opportunity announcements to solicit innovative research on artificial pancreas systems by small businesses. The NIDDK has also participated in the NICHD-led Diabetes Research in Children Network, or DirecNet, which has investigated the use of technological advances in the management of type 1 diabetes in children and adolescents. As a result of its own efforts and these collaborations, the NIDDK is currently supporting a multi-faceted program of research at academic centers and small businesses to:

- Conduct clinical studies of portable artificial pancreas devices;
- Make stable, fast-acting formulations of insulin and another hormone important to managing blood glucose levels, glucagon;
- Improve glucose sensors' sensitivity and durability;
- Build and test devices that combine sensing and hormone delivery;
- Study physiological and behavioral factors to make artificial pancreas use easier; and
- Develop the next generation of glucose-sensitive technology.

Through recent initiatives, the NIDDK is also fostering research training of bioengineers and development of an interdisciplinary workforce to develop innovative technologies for diabetes treatment, including artificial pancreas systems. Notably, a significant portion of NIH-supported artificial pancreas research over the past 15 years has been made possible by funds from the *Special Statutory Funding Program for Type 1 Diabetes Research*.

JDRF supports its own program of research on the artificial pancreas, the Artificial Pancreas Project, which it launched in 2006. Over the years, the NIDDK has increased collaboration with JDRF and other

important funding entities, such as The Leona M. and Harry B. Helmsley Charitable Trust, to coordinate efforts so that there can be more efficient investment of resources to expedite advances in this field. For example, the NIDDK and JDRF are both funding sites in the Artificial Pancreas Consortium, a multi-site, international consortium of clinicians, engineers, and mathematicians who have tested early, hospital-based versions of an artificial pancreas and have started to test outpatient, wearable systems. Moreover, the NIDDK and JDRF have organized scientific panels of artificial pancreas investigators at the American Diabetes Association's annual Scientific Sessions, with the next one planned for June 2014.

The collaborative workshops organized by the NIDDK, the FDA, and JDRF, as well as the NIDDK and JDRF initiatives, have also inspired investment in artificial pancreas technologies by other, private foundations. For example, The Leona M. and Harry B. Helmsley Charitable Trust has recently launched initiatives on the artificial pancreas, such as the Trust's Automated Insulin Delivery Initiative. The Trust also funds the "bionic pancreas" project (as described later).

As a result of research investments and collaborative activities to promote artificial pancreas technologies, there has been rapid progress in the past few years toward achieving effective, wearable systems. For example, in just the last year:

- *"Low glucose suspend" devices:* The objective of these devices is to temporarily shut off insulin delivery when circulating glucose levels fall below a set threshold, to help prevent episodes of dangerously low blood glucose levels—an important aspect of artificial pancreas functionality. A recently published clinical study supported by industry (Medtronic) demonstrated that hypoglycemia events were less frequent, and events at night were less severe and shorter in duration, in patients using a glucose-sensor augmented low glucose suspend device at home. In September 2013, the FDA approved the Medtronic Minimed 530G, the device that was used in this trial, for use by people with diabetes 16 years of age and older. This is the first example of an artificial pancreas technology approved under the FDA guidance released in November 2012.
- *"Diabetes Assistant (DiAs)":* A number of investigators are developing and testing systems that close the loop between glucose sensing and insulin delivery using smartphone based technology, which would make the system practical for use outside of a hospital setting. A recently published study of one such system, the DiAs, which uses an Android-based smartphone, has demonstrated the feasibility of its use to control blood glucose levels in a hotel-based outpatient setting. This effort has been supported by the NIDDK and JDRF *(see also "Smartphone Technology Advances Progress Toward the Development of an Artificial Pancreas for People with Type 1 Diabetes" in this chapter).*
- *Bihormonal bionic pancreas:* To more fully recapitulate the hormonal controls of blood glucose levels lost in type 1 diabetes, some researchers are already working on systems that include both insulin, which lowers blood glucose levels, and glucagon, which raises them. Researchers working on a bihormonal bionic artificial pancreas based on an iPhone platform recently completed two outpatient studies in adults and children, with encouraging results. The "bionic pancreas" effort has received support from the NIDDK, JDRF, and The Leona M. and Harry B. Helmsley Charitable Trust, among others.

In concert with academia and industry, the NIDDK/NIH, the FDA, and JDRF have collectively catalyzed very intense and productive activity in the field of artificial pancreas research. This joint commitment by public and private institutions to an active role in the development of an artificial pancreas has fostered remarkable progress in recent years. Looking ahead, it is anticipated that these collaborative efforts will continue to lead to new successes in harnessing this new technology to improve the lives of people with diabetes.

Nobel Prize Honors Discoveries About the Transport System in Cells

Dr. James E. Rothman, 2013 Nobel Laureate.
Photo credit: Harold Shapiro, Yale University.

One of the winners of the 2013 Nobel Prize in Physiology or Medicine is Dr. James E. Rothman, an NIDDK grantee. Currently a professor at Yale University, Dr. Rothman has received funding from several NIH Institutes, and much of the research that earned him the Nobel Prize was supported by a longstanding NIDDK grant that began in 1980. He has served on NIDDK's National Advisory Council.

He shares the Nobel Prize with two other scientists, Drs. Randy W. Schekman and Thomas C. Südhof, also both NIH grantees who have received funding from other Institutes.

Dr. Rothman was honored for his discovery of the machinery that regulates the traffic of small membrane-enclosed shuttles, called vesicles, which transport molecular cargo from one compartment within a cell to another, and between a cell and its environment. Exquisitely organized, this vesicle transport system underlies extraordinarily diverse biological processes critical for health, as vesicles carry hormones, signaling molecules for the nervous system, and other vital cargo. Defects in the process are associated with diabetes, neurologic diseases, and other adverse health conditions. Dr. Rothman's research is notable both for his landmark discoveries and for the pioneering experimental approach he took amidst an atmosphere of skepticism decades ago. His strategy was to break open cells and mix various cellular materials back together to try to reconstitute transport in a test tube; if that worked, he could then purify the necessary components of the transport machinery from the mixture to learn their identity. Many scientists at the time, however, believed that vesicles, outside the orderly confines of a cell, would not find the right target destination. But the approach worked, and Dr. Rothman and members of his laboratory went on to identify the machinery integral to this process, including a group of proteins they called SNAREs. Embedded in the membranes that surround vesicles and other cellular compartments, different SNAREs interact selectively so that vesicles dock at the correct destination. The SNAREs then promote fusion of the vesicle and target membranes, allowing release of the cargo. Since his groundbreaking findings years ago, Dr. Rothman's research has continued to yield new insights into this essential biological transport system.

The three scientists presented their Nobel Lectures in Stockholm on December 7, and received their awards on December 10, 2013.

NIDDK Scientist Recognized for Outstanding Contributions to Diabetes Research and Mentorship

NIDDK Director Dr. Griffin P. Rodgers (left) and Dr. Peter H. Bennett (right) at Dr. Bennett's NIH Grand Rounds lecture in October 2013. ***Photo credit: Maria Maslennikov.***

Dr. Peter H. Bennett accepts the first Harold Hamm International Prize for Biomedical Research in Diabetes in October 2013. ***Photo courtesy of the Harold Hamm Diabetes Center.***

Scientist Emeritus and former Chief of the NIDDK intramural Phoenix Epidemiology and Clinical Research Branch (PECRB), Dr. Peter H. Bennett, received two prestigious awards in 2013 in recognition of his enormous impact on the field of diabetes research and his training and mentorship of diabetes investigators.

In recognition of his many accomplishments, on October 28, 2013, Dr. Bennett was the inaugural recipient of the $250,000 Harold Hamm International Prize for Biomedical Research in Diabetes. This prize recognizes innovation in the field of diabetes research with an emphasis on progress toward a cure. Awarded by the Harold Hamm Diabetes Center at the University of Oklahoma, the prize celebrates the scientific achievements of an outstanding researcher, team of researchers, or research institution selected by a rotating jury of national and international leaders in the diabetes community. Dr. Bennett also received the American Diabetes Association (ADA) 2013 Albert Renold Award at the ADA's 73rd Scientific Sessions in June 2013. The award is one of the ADA's highest scientific honors, given annually to an individual who has made a significant impact as a mentor of diabetes researchers and/or as a facilitator of a community of diabetes investigators. In addition to establishing a robust research program within the PECRB and providing training to hundreds of investigators worldwide, Dr. Bennett has directly mentored more than 50 scientists, many of whom now hold leadership positions in academic and federal institutions focused on diabetes and its complications.

Dr. Bennett's work on type 2 diabetes began in the 1960s, a time when little was known about the disease and nothing was known about its risk factors. Following his medical education at Victoria University of Manchester, United Kingdom, Dr. Bennett joined the NIDDK, which began conducting cooperative research on diabetes with the Gila River Indian Community in 1965, after scientists discovered the high prevalence of type 2 diabetes in this population. For over 30 years, glucose tolerance tests were given to almost every Pima Indian five years of age or older every two years. Results showed the Arizona Pima Indians had the highest prevalence of type 2 diabetes of any group in the world—including the genetically similar Mexican Pima Indians—leading Dr. Bennett to conclude that

environmental factors play a role in the prevalence of type 2 diabetes. As this multi-faceted study moved forward, he found that low physical activity and obesity were risk factors for the disease. He identified insulin resistance as another risk factor. Dr. Bennett's research led directly to new, evidence-based criteria for diagnosing type 2 diabetes and a high-risk prediabetic state, impaired glucose tolerance. All of these findings applied far beyond the Pima population, dramatically changing the diabetes landscape and setting the stage for clinical trials to prevent or delay type 2 diabetes in people at high risk, such as the landmark NIDDK-led Diabetes Prevention Program.

Through the long-running study in partnership with the Pima Indians, Dr. Bennett also found that children whose parents had diabetes were more likely to develop diabetes earlier in life and that breastfeeding prevented the early development of diabetes. Later, it emerged that diabetes during pregnancy conferred a greatly increased risk of early-onset diabetes to offspring—a finding that has been especially important to improving management of diabetes and its risk factors for mothers and their children, and which helped pave the way to ongoing studies of epigenetic factors during intrauterine development that predispose the offspring to obesity, type 2 diabetes, and other metabolic problems later in life.

Dr. Bennett's achievements in the field of diabetes research continue to be the foundation for the prevention, diagnosis, and treatment of type 2 diabetes and its complications. According to Dr. Clifton Bogardus, his long-time colleague and current Chief of the PECRB, "The contributions made by Dr. Bennett, in close collaboration with the Gila River Indian Community, to the understanding of the etiology and pathogenesis of type 2 diabetes have greatly influenced methods for the prevention and treatment of this disease worldwide and cannot be overstated. Equally important, he has trained and mentored countless numbers of scientists both within the NIDDK and elsewhere that will have a lasting legacy in the field of metabolism for decades. He was able to make these extraordinary achievements not just due to his great intellect and scientific acumen, but also due to his humility, collegiality, and cultural sensitivity that made it possible to work closely with the Pimas and collaborators around the world."

This feature was adapted from an article by Mr. Eric Bock that originally appeared in the January 2014 edition of the NIH Record.

NIDDK Director Testifies on Type 1 Diabetes Research

On July 10, 2013, NIDDK Director Dr. Griffin P. Rodgers testified about progress in type 1 diabetes research before the Senate Special Committee on Aging, which is led by Chairman Bill Nelson (D-FL) and Ranking Member Susan Collins (R-ME). The hearing, entitled "Diabetes Research: Reducing the Burden of Diabetes at All Ages and Stages," was held in conjunction with the Children's Congress, an event sponsored every two years by JDRF (formerly the Juvenile Diabetes Research Foundation). In his testimony, Dr. Rodgers described research made possible by the *Special Statutory Funding Program for Type 1 Diabetes Research*, including progress from clinical trials testing approaches to delay or prevent type 1 diabetes, and progress toward the development of an artificial pancreas—a device to automate blood glucose sensing and insulin administration. The NIDDK administers the *Special Program* on behalf of the HHS Secretary.

Testifying with Dr. Rodgers were National Basketball Association star Ray Allen and his six-year-old son, Walker, who was diagnosed with type 1 diabetes at 17 months of age; Emmy Award-winning actress Jean Smart, who was diagnosed with the disease at age 13; JDRF President and CEO Jeffrey Brewer, also the father of a child with type 1 diabetes; and 14-year-old Quinn Ferguson, a JDRF Children's Congress delegate and participant in an NIDDK-supported clinical trial being conducted by Type 1 Diabetes TrialNet.

Shown at dais (left to right): Sen. Susan Collins (R-ME), Sen. Bill Nelson (D-FL), Sen. Elizabeth Warren (D-MA), Sen. Joe Donnelly (D-IN), and Sen. Jeanne Shaheen (D-NH). *Photo copyright: James T. Murray.*

Shown at table (left to right): Jean Smart, Walker Allen, Ray Allen, Dr. Griffin P. Rodgers, Jeffrey Brewer, and Quinn Ferguson. JDRF Children's Congress delegates sit in the foreground. *Photo copyright: Larry Lettera.*

NIDDK Director Dr. Griffin P. Rodgers. *Photo copyright: James T. Murray.*

Sen. Susan Collins and Sen. Bill Nelson. *Photo copyright: Geoff Hauschild.*

Sen. Joe Donnelly and Sen. Jeanne Shaheen. *Photo copyright: Geoff Hauschild.*

STORY OF DISCOVERY

The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions And Complications Study: Thirty Years of Research That Has Improved the Lives of People with Type 1 Diabetes

Diabetes slowly damages major organs in the body, such as the eyes, kidneys, and heart. Impressive research progress toward combating diabetes complications was achieved through a large clinical trial launched by the NIDDK in 1983. The Diabetes Control and Complications Trial (DCCT) was a multi-center clinical trial in 1,441 people ages 13 to 39 years with type 1 diabetes. It compared the effects of intensive versus conventional treatment of blood glucose levels on the development of microvascular complications (those affecting the small blood vessels in the eyes, kidneys, and nerves). Participants in the intensive treatment group kept their blood glucose levels and hemoglobin A1C (HbA1c) levels—which reflect average blood glucose levels over a two- to three-month period—as close to normal as safely possible through a regimen that included frequent monitoring of blood glucose and at least three insulin injections per day or use of an insulin pump. The study's conventional treatment, based on what was standard practice at the time the DCCT began, consisted of one or two insulin injections per day, with once-a-day urine or blood glucose testing.

The two treatment groups achieved markedly different average HbA1c levels over the course of the trial, and strikingly different rates of microvascular complications. The DCCT proved conclusively that intensive therapy reduces the risk of microvascular complications, such as diabetic eye, kidney, and nerve damage, by 35 to 76 percent compared with what was then conventional treatment. It also demonstrated that HbA1c measurements could be used by health care providers and patients to monitor disease management by assessing blood glucose control and predicting risk of developing complications. The DCCT findings had a profound impact on clinical practice for the management of type 1 diabetes by leading to the development of clinical guidelines to recommend HbA1c targets for people with the disease; it also spurred the creation of the National Diabetes Education Program, co-led by the NIDDK and the Centers for Disease Control and Prevention (CDC), to disseminate the findings to patients and health care providers (*www.ndep.nih.gov*).

Long-term Benefits of Intensive Blood Glucose Control

Upon completion of the DCCT, participants who had received conventional treatment were taught the intensive treatment methods, and all were encouraged to use intensive treatment on their own, although the intervention itself stopped. Nearly all people who participated in the DCCT volunteered for the follow-on Epidemiology of Diabetes Interventions and Complications (EDIC) study, which began in 1994. EDIC was established to determine the long-term outcomes of reducing exposure of the body's tissues and organs to high blood glucose levels.

STORY OF DISCOVERY

About five years after the transition to EDIC, blood glucose control (as measured by HbA1c levels) in the former intensive and conventional treatment groups converged to a similar level. This convergence occurred because of changes in glucose control in both groups. The level of blood glucose control in the participants from the former intensive treatment group was not quite as tight as it was during the DCCT, when they had the advantages of the clinical trial setting—although they were still able to achieve better glucose control on their own after the DCCT than they had before the trial. At the same time, those who were in the conventional treatment group during the DCCT began implementing a more intensive control regimen afterward, and thus improved their glucose control. With both groups now striving for intensive control on their own, the net result was that blood glucose control became nearly identical in the two groups during EDIC.

In 2002 and 2003, DCCT/EDIC investigators reported that, even though the two treatment groups had similar blood glucose control during EDIC, the former intensive treatment group had long-term health benefits from the finite period (averaging six and a half years) of intensive glucose control during DCCT: they continued to have reduced risk for microvascular complications seven to eight years after the end of DCCT compared to the former conventional treatment group. The phenomenon of long-lasting effects of a period of intensive or non-intensive glucose control has been termed "metabolic memory." More recent observations show that the differences in new cases of diabetic eye disease between participants who received the intensive treatment and those who received conventional treatment are beginning to narrow. Nonetheless, three decades after the start of DCCT, the two former treatment groups have substantially different rates of complications, suggesting that people with type 1 diabetes implement intensive glucose control as early in the course of the disease as possible.

An important unanswered research question after the DCCT ended was the effect of glucose control on cardiovascular disease (CVD), as CVD can take a long time to develop and the participants were too young to examine this complication during the DCCT. That question was answered in 2005—over 20 years from the start of the trial—when the DCCT/EDIC research group reported that intensive blood glucose control reduced the risk of nonfatal heart attack, stroke, or death due to CVD by 57 percent. These results showed for the first time that intensive control of blood glucose levels has long-term beneficial effects on CVD risk in people with type 1 diabetes. These findings are particularly significant because people with type 1 diabetes face a 10-fold increased risk of CVD death compared to the general age-matched population.[1,2]

Insights continue to emerge regarding the long-term benefits of early and intensive blood glucose control. In 2009, DCCT/EDIC researchers found that, after 30 years of diabetes, DCCT participants in the former intensive treatment group had about half the rate of eye damage compared to those in the former conventional treatment group (21 percent versus 50 percent). They also had lower rates of kidney damage (9 percent versus 25 percent) and cardiovascular events (9 percent versus 14 percent) compared to those in the former conventional treatment group. These findings suggest that with early intensive therapy to control blood glucose levels, the outlook for people with type 1 diabetes is better than ever.

More good news was reported in 2011 related to the long-term risk of developing kidney disease. During the DCCT, the participants were too young for researchers to examine the effect of glucose control on actual kidney disease, which (like CVD) can take a long time to develop. The researchers did find that at the end of DCCT, intensive therapy reduced a condition associated

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with kidney damage, called albuminuria. However, following the participants for longer timeframes in EDIC allowed the researchers to examine the development of kidney disease. In 2011, after an average 22-year follow up, DCCT/EDIC demonstrated that controlling blood glucose early in the course of disease not only continued to reduce albuminuria, but also decreased participants' long-term risk of developing kidney disease by 50 percent. This important finding showed that controlling blood glucose early in the course of type 1 diabetes yields huge dividends, preserving kidney function for decades.

In 2013, the DCCT/EDIC researchers and patient volunteers celebrated a remarkable 30 years of participation in research since the launch of the DCCT in 1983. To date, 95 percent of living DCCT patient volunteers continue to participate in EDIC. This unwavering dedication to research has transformed how type 1 diabetes is treated: when people with the disease visit their doctors today, the advice that they receive stems directly from DCCT/EDIC research findings—a testament to the impact that DCCT/EDIC research has had on a public health level.

Research To Combat Hypoglycemia and To Help People Achieve Recommended Levels of Blood Glucose Control

Even though the results of DCCT/EDIC show that intensive glucose control is beneficial for long-term prevention of complications, type 1 diabetes is a burdensome disease to manage. It requires patients (or parents of young children) to check their blood glucose levels with finger sticks, monitor food intake and physical activity levels, and administer insulin. In addition, a severe limitation to the practice of intensive therapy is the potential for acute episodes of hypoglycemia, or low blood glucose. The immediate effects of hypoglycemia can be severe, including changes in cardiovascular and central nervous system function, cognitive impairment, increased risk for unintentional injury, coma, and death. Therefore, the DCCT/EDIC findings underscore the importance of research to develop new tools to help patients achieve recommended levels of glucose control with less risk of hypoglycemia.

NIDDK-supported researchers have already been successful in contributing to the development of U.S. Food and Drug Administration (FDA)-approved continuous glucose monitoring technology, which may enable better glucose control by providing patients with real-time measurements of glucose levels every few minutes and sounding alarms when glucose levels are too high or too low. The NIDDK also supports research to develop artificial pancreas technology, which would integrate continuous glucose monitoring with automated insulin delivery based on the glucose data, representing an important current opportunity to help people with diabetes implement intensive blood glucose control. Other research is pursuing strategies to develop approaches to replace or regenerate the body's insulin-producing beta cells, which are lost in type 1 diabetes, as a potential cure for the disease. Through these multi-faceted approaches, the NIDDK remains committed to helping people with type 1 diabetes safely achieve good blood glucose control with less burden, to realize the full benefits of the DCCT/EDIC findings.

Benefits for Type 1 Diabetes and Beyond

Findings from the DCCT/EDIC have transformed the management of type 1 diabetes, but have also benefitted people with type 2 diabetes. For example, the results of DCCT/EDIC stimulated the conduct of trials assessing the role of blood glucose control in type 2 diabetes. These trials have informed clinical guidelines developed by the American Diabetes Association and other groups, which now include HbA1c targets for most people with diabetes.

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However, lack of standardization of HbA1c tests made it difficult to utilize these targets in medical practice. In other words, there were many different types of HbA1c tests being used that gave varied results, so results were not comparable from one laboratory to another. To address this gap, the NIDDK and the CDC launched the National Glycohemoglobin Standardization Program in 1996 to improve the standardization and reliability in measures of HbA1c. The standardization effort has been a great success: variability of HbA1c test results has steadily reduced, and the availability of a standardized testing method has allowed international experts to recommend expanding the use of HbA1c beyond monitoring of blood glucose control during treatment to use it as a more convenient test to diagnose type 2 diabetes. Thus, standardization of the HbA1c test has not only benefited management of type 1 and type 2 diabetes by enabling health care providers and patients to accurately and meaningfully assess blood glucose control and risk for complications, but has expanded the utility of the HbA1c test to be used for diagnosis of type 2 diabetes.

In addition, the DCCT established the value of HbA1c as an outcome measure for clinical trials in both type 1 and type 2 diabetes. This has dramatically shortened the cost and duration of trials for new therapies because improvements in HbA1c levels are detected long before changes in complications become apparent. The use of HbA1c as an outcome measure was the basis for FDA approval of approximately 10 new classes of drugs for type 2 diabetes, as well as for improved forms of insulin. These are just a few examples of the far-reaching benefits that have stemmed from DCCT/EDIC research.

A Long-term Investment in Research Improves the Lives of People with Type 1 Diabetes

The DCCT/EDIC demonstrates how a long-term investment in research has had a profound impact on the health of people with type 1 diabetes. Thirty years after the beginning of the DCCT, researchers are still demonstrating significant findings that continue to improve the care of people with type 1 diabetes and also have implications for people with type 2 diabetes. Because the cohort of DCCT patients was too young for examination of cardiovascular complications and kidney disease when the study began, the long-term follow up was necessary to assess the effect of intensive glucose control on these devastating and life-threatening diabetic complications. The research shows that the full benefits of treatment may not be seen for decades, especially for complications of diabetes, which can progress slowly but have devastating consequences. It has only been through 30 years of steadfast dedication of the DCCT/EDIC research group and patient volunteers that these benefits continue to emerge. They are a key reason why people with type 1 diabetes are living longer, healthier lives than ever before.

[1] *Krolewski AS, Kosinski EJ, Warram JH, et al. Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. Am J Cardiol 59: 750-755, 1987.*

[2] *Dorman JS, Laporte RE, Kuller LH, et al. The Pittsburgh insulin-dependent diabetes mellitus (IDDM) morbidity and mortality study: mortality results. Diabetes 33: 271-276, 1984.*

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Genomics of Type 2 Diabetes and Metabolic Syndrome: Lessons from a Founder Population

Dr. Alan R. Shuldiner

Dr. Alan R. Shuldiner is the John L. Whitehurst Professor, Associate Dean for Personalized Medicine, and Director of the interdepartmental Program for Personalized and Genomic Medicine at the University of Maryland School of Medicine, as well as co-Director of the University of Maryland Clinical and Translational Research Institute. He received his M.D. from Harvard Medical School, and completed his residency in internal medicine at Columbia-Presbyterian Hospital in New York, later becoming a Medical and Senior Staff Fellow in Endocrinology and Metabolism in the NIDDK's Intramural Research Program. Through projects in many areas, and in particular through genomic research with the Old Order Amish of Pennsylvania, Dr. Shuldiner has made important contributions to understanding the molecular causes of type 2 diabetes and cardiovascular disease. In his presentation, he described some of the discoveries that have stemmed from his work with the Amish.

Studying Chronic Disease in the Old Order Amish

Dr. Shuldiner began by recognizing the multidisciplinary team of scientists with whom he works. Describing his research focus, he introduced the interaction among genes and the environment in the development of type 2 diabetes; cardiovascular disease (heart disease and stroke); and the metabolic syndrome, a constellation of cardiovascular risk factors, which includes insulin resistance and diabetes. He then turned to his studies of the genetics of these complex diseases, highlighting his longstanding partnership with the Old Order Amish community, whose participation has made this research possible.

Explaining that the Amish are an excellent population for studying complex genetic metabolic diseases, he described attributes of their lifestyle and genetics that have facilitated this research. They have a traditional lifestyle that has changed little since the 18th century, with low use of medications, no cars, television, telephones, or even electricity; and they rely on manual labor and a diet composed largely, though not exclusively, of foods grown and prepared within the community. This relatively homogeneous lifestyle helps to minimize some problems common to other large genomic studies: the potential that any particular gene variant might have variable effects among people with widely differing exposure to environmental factors that contribute to or protect from chronic disease.

Moreover, the Amish represent a "closed founder community," which is to say they began as a relatively small population who immigrated to this country and, as their population has grown, they have remained cohesive, with little or no intermarriage with outside groups. Thus, while there is genetic variation among the Amish, they have less overall genetic complexity than larger groups. Among people in a more heterogeneous population (all Americans of European descent, for example), there may be many variations in a particular, important gene: some conferring disease risk, some conferring disease protection, and some that have no effect. Thus, the genetic homogeneity of the Amish simplifies the process of identifying disease-affecting genetic differences among them in two ways. First, high disease risk variants that are very rare in the general population may have increased in

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frequency in the Amish, making them easier to identify and study. Second, most of the common variants that have a smaller effect on disease risk that are present in the general population also exist in the Amish. These risk alleles may have more consistent effects in populations that are more homogeneous with respect to genetics and lifestyle, such as the Amish.

Because Amish families typically have many children, researchers can more easily detect inheritance patterns of genetic traits. Family members also tend to live close to each other, and this proximity has enabled Dr. Shuldiner's team to recruit several thousand Amish individuals as participants in the research. Another characteristic of the Amish—that they have maintained extensive genealogical records dating to the 18th century—has been of great value to genetic research by describing how community members are related to one another, and helping to determine the lifetime impact of specific traits.

Dr. Shuldiner noted that his work has also benefitted from the University of Maryland Amish Research Clinic, a state-of-the-art facility that he helped establish in the heart of Amish Country in Pennsylvania, which has greatly enhanced collaboration between researchers and the Amish people, and has been instrumental for making the discoveries outlined below.

A Gene with a Profound Effect on a Key Class of Fats in the Blood

Thanks to the first line of research Dr. Shuldiner described, we now know it may one day be possible to treat or stave off coronary artery disease by inhibiting the action of a factor in blood involved in transport of certain fats in the body. The body stores most fat in chemical compounds called triglycerides. Both high fasting levels of triglycerides and poor clearance of triglycerides after a high-fat meal are known to be risk factors for heart attacks and stroke; and these fasting and post-meal triglyceride levels vary considerably among different people, an observation long thought to be at least partially explained by genetic variation. Dr. Shuldiner described how studying the Amish bolstered this hypothesis through the discovery of a mutation in one of the triglyceride carrier proteins.

After giving a set amount of a milkshake-like high-fat drink to more than 800 Amish men and women, Dr. Shuldiner and colleagues measured the beverage's effect on blood triglyceride levels over the next few hours. The researchers found considerable variation in the resulting triglyceride load in blood samples from different participants—just as would have been expected in a less homogeneous population—suggesting that some individuals in the Amish population may harbor genes that promote rapid clearance of dietary fat, while the genes of other Amish people may lead to average or slower triglyceride clearance.

The researchers then used a powerful genetic and computational method—a genome-wide association study—to probe the participants' genomes for gene variants that correlated with very good or very poor triglyceride clearance. In this way, they discovered that a genetic variation (mutation) that inactivates the gene *APOC3* was strongly associated with low triglyceride levels. Most Amish people—and most everyone else—have two working copies of *APOC3*. Close examination, however, revealed that about five percent of the Amish have the triglyceride-lowering mutation identified through this study in one of their *APOC3* gene copies. (The mutation is much rarer among the non-Amish.) Because triglycerides, like other fats, are not soluble in water, the body utilizes special carriers called lipoproteins for moving triglycerides in the bloodstream. One of the triglyceride-carrier molecules, Apo C-III, is

the product of the *APOC3* gene. As might be expected, having one normal and one mutant *APOC3* effectively cuts in half the amount of Apo C-III and triglycerides in the blood stream.

Rather than conferring an illness, as many mutations in other genes do, this *APOC3* mutation seems to help those who have it maintain healthier serum triglyceride levels. In fact, the mutation also seems to inhibit development of another marker of heart disease risk, calcification of the coronary arteries. However, despite these apparent benefits, it remained unclear whether the mutation helps those who have it live longer. To get a better handle on the mutation's overall effect on health, the researchers turned to the extensive birth and death records maintained by the Amish. They found that the mutation actually seems to help extend life: almost 1 in 4 Amish people inferred to have had a copy of the *APOC3* mutation lived to be at least 90, compared to less than 1 in 10 of those who lacked it.

The fact that these observations—that having low Apo C-III levels reduces heart disease risk factors and that it seems to lengthen life—were made in a human population suggests that a pharmaceutical approach to inhibit Apo C-III action or to lower the protein's levels may be safe and effective for treating or preventing heart disease. Indeed, Dr. Shuldiner noted that another team of researchers, at a pharmaceutical company, recently published the results of a preliminary, short-term test of one method for lowering Apo C-III, in which they showed significant reduction of the participants' triglyceride levels. Further research will be needed to determine whether the approach is safe and effective for prevention or treatment of cardiovascular disease in long-term use, and whether different diets may be healthy for different people, depending on their genetics—an important example of the emerging field of "nutrigenomics."

A Mutation That Renders an Otherwise Valuable Medicine Ineffective for Some People

Dr. Shuldiner next described research that led to the discovery of a mutation that keeps many people from benefiting from clopidogrel (brand name Plavix™), a drug prescribed to help prevent recurrence of heart attacks (myocardial infarctions) and strokes in people who have experienced such an event. Heart attacks and strokes often result from clots that form inside blood vessels, limiting blood flow to the heart or the brain, respectively. Clots are formed by blood cells called platelets, and are critical for staunching blood flow from cuts and scrapes. Clopidogrel binds to a specific protein on the surface of platelets, preventing them from aggregating to form a clot and thus reducing the occurrence of undesirable clotting within the blood stream that can lead to a heart attack or stroke. In most people, clopidogrel does this quite effectively. But there is considerable variation in the capacity of the drug to inhibit platelet aggregation in different individuals, so that some people seem not to benefit from the medication.

Dr. Shuldiner and his colleagues measured platelet aggregation in more than 650 Amish volunteers before and after one week of clopidogrel therapy, and found that the variable nature of the clopidogrel response is also seen among the Amish: some had a strong response to clopidogrel, while in others the medicine had almost no impact on clotting. Through a statistical approach that again allowed them to utilize information from the Amish genealogical records to determine how the participants were related to one another, the researchers were able to determine that about 70 percent of the variation in clopidogrel response was the result of genetic factors. This observation suggests that if the genes affecting the clopidogrel response can be identified, it might be beneficial to employ a "pharmacogenomic" treatment

strategy—using genetic tests to determine whether clopidogrel or newer, more costly anti-platelet drugs will be best for a particular patient.

By utilizing another genome-wide association approach, Dr. Shuldiner and his fellow researchers were able to identify one such gene, *CYP2C19*, and a mutation in the gene that makes clopidogrel dramatically less effective. In the absence of clopidogrel, they found that platelet function is normal whether or not someone has the mutation. But people with one normal and one mutant *CYP2C19* gene responded significantly less strongly to the medicine than those with two normal copies; and in people with two mutant copies of the gene, clopidogrel had almost no effect. Based on pharmacological studies of others, it was discovered that the normal product of the *CYP2C19* gene is a protein that converts clopidogrel from a biologically inactive compound into its effective form. Thus, in people lacking the protein, clopidogrel remains effectively inert, and in people with half as much of the protein as others, an intermediate amount of the medicine is converted into its active, platelet-inhibiting form.

Unlike the *APOC3* mutation, which is extremely rare among the non-Amish, the *CYP2C19* mutation is fairly common: about one-third to one-half of non-Amish individuals have at least one copy, and have about a 2.4-fold higher risk of heart attack or stroke than a person with no *CYP2C19* mutation taking clopidogrel.

The U.S. Food and Drug Administration responded to these findings and similar findings by other researchers by requiring clopidogrel manufacturers to change the labeling on the medication's packaging to note this effect, as well as the fact that tests for the presence of the *CYP2C19* mutation are available, and that other drugs may be more effective for people who have it.

Dr. Shuldiner noted that as significant an effect as the mutation has, however, it accounts for just 12 percent of the overall variation in clopidogrel-induced changes in blood clotting, so more work is needed to identify other mediators of patient response to the medicine. Because the drug is highly effective for preventing heart attack and stroke in most people at high risk for such events, and is also safe and inexpensive relative to alternatives, clopidogrel—along with low-dose aspirin—remains a widespread and highly beneficial anti-platelet therapy for most people at high risk of heart attack or stroke.

Conclusion: Type 2 Diabetes and the Old Order Amish

In ongoing research, Dr. Shuldiner and colleagues are shedding new light on the genetic underpinnings of type 2 diabetes. Through their Amish Family Diabetes Study and other epidemiological research, they found that Amish children tend to be leaner and more physically active than other American children, but that Amish adults are about as likely as other Americans to be obese. Furthermore, while the Amish are actually more likely than other groups to have prediabetes, they are less likely to develop type 2 diabetes. A reasonable hypothesis to explain these findings is that the Amish population harbors significant genetic risk for type 2 diabetes, but that their physically active lifestyle offers them some protection from the disease.

Indeed, genome-wide analysis revealed evidence that genetic variation among the Amish does markedly affect the probability of developing type 2 diabetes, and it is enticing to speculate that one or more genetic variants found to be associated with type 2 diabetes among the Amish might have particularly potent effects, since they would be capable of overcoming the protective qualities of the active Amish lifestyle.

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Going forward, the team of scientists aims to elucidate the physiological basis for these variations in risk.

Dr. Shuldiner concluded by discussing how the analysis of such genetic variants in the Amish can often shed light on the molecular mechanisms of metabolic regulation in all people, help optimize treatment approaches for type 2 diabetes and cardiovascular disease in individual patients, and suggest potential new targets for therapeutic intervention.

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Wesley Wilson

A Lifetime of Type 1 Diabetes May Hold a Key to the Future

Wesley Wilson and his family. Left to right: Kate, Patrick, Justin, Luella, Wesley, Beth, Roger, and Kathleen.

Wesley Wilson has a lot to celebrate these days. He and his wife recently celebrated their 80th birthdays with their family, friends, and Wesley's doctors. Although he jokingly admits to wearing out some of the doctors he's seen since moving to Montana in 1963, Wesley's doctors are welcome fixtures at his parties, especially at the one celebrating his receipt of the Joslin 50-Year Medal, an award for living with type 1 diabetes for 50 years. "When I got my 50-Year Medal, we had a big party. It was nice to have something to celebrate in regards to diabetes," says Wesley.

Type 1 diabetes is an autoimmune disease in which the immune system destroys the cells in the pancreas that make insulin. People with the disease must carefully monitor blood sugar levels and administer insulin, either through injections or an insulin pump. Despite vigilance in management of their diabetes, it is difficult for people to achieve near normal blood sugar levels and to prevent the complications of the disease.

The Joslin Medalist Program at the Joslin Diabetes Center in Boston, Massachusetts, began awarding medals to people with type 1 diabetes in 1948, with the hope that they would serve as incentive to those committed to good, albeit challenging, diabetes care. At first, the Program awarded 25-Year Medals but, with improvements in care and treatment, Joslin was able to begin awarding the 50-Year Medal in 1970, the 75-Year Medal in 1996, and the 80-Year Medal in 2013. To date, Joslin has presented over 4,000 50-Year Medals, 68 75-Year Medals, and 4 80-Year Medals. Given the challenges that living with type 1 diabetes provides and the devastating complications that it can cause, these medalists are a special group to be celebrated.

"It was nice to have something to celebrate in regards to diabetes," says Wesley, of his Joslin 50-Year Medal.

The Doctor Becomes a Patient

Wesley was diagnosed with type 1 diabetes during his second year of medical school in 1956 and had been married just a few months earlier. "We got married in July and in the fall, my wife said you've been drinking a lot of water and urinating an awful lot." As a young medical student, Wesley hadn't had clinical experience, but his wife, who was a nurse at the time, encouraged him to go to a doctor. His blood sugar was high, and he was

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diagnosed with type 1 diabetes. Wesley recalls, "I was so involved with med school that, when I think back on it, it didn't seem to make a great impression on me. I mean I did realize that I had to change my lifestyle and take insulin, but I was just kind of going to sail along."

Then, in December, his class began to study pathology, and the lectures focused on diabetes and its complications. To this day he remembers those lectures very well. "They [the lectures] are imprinted indelibly in my head. At that time it was taught that folks with diabetes had a life expectancy about a third as long as the usual population and then, of course, it was always marked by complications." Wesley remembers being taught that, "there was nothing you could do to prevent eye, kidney, and heart disease. So the lecture had more of an impact on me than just the initial diagnosis, including that whatever you did didn't make much difference."

But it wasn't long before Wesley was introduced to the idea that what he did could make a difference. He began his residency at the University of Oregon and, once again, credits his wife because she was working at a hospital linked to the Portland Diabetes Center. A doctor there had trained at the Joslin Diabetes Center and was a believer in tight sugar control even before blood sugar testing technology became available. "We had classes on carbohydrates and how to adjust the insulin to try to match the carbohydrate content in the meal. I think that was the first effort to try and achieve some degree of control," remembers Wesley.

Subsequent research has shown that Wesley's doctor was correct about the importance of tight sugar control—the NIDDK's Diabetes Control and Complications Trial (DCCT) demonstrated in 1993 that achieving blood sugar control as close to normal as safely possible reduced the risk of eye, kidney, and nerve complications associated with type 1 diabetes. In 2005, the DCCT's follow-on study, the Epidemiology of Diabetes Interventions and Complications, demonstrated that intensive blood sugar control could also reduce the risk of cardiovascular disease-related complications. These results revolutionized treatment of type 1 diabetes and greatly improved the prognosis of people with the disease.

The Joslin 50-Year Medalist Study

Wesley has seen the complications of diabetes; after training in hematology—the study of blood—and oncology, he switched his specialty to diabetes, after the DCCT showed tight sugar control could reduce the risk of complications, providing care to people with type 1 or type 2 diabetes. Fortunately, Wesley has not had to deal with these complications as a result of his own type 1 diabetes, something that amazes him to this day. "To be able to go 50 years without really any significant complications is a little bit hard for me to believe. It's worth the celebration just for that reason." In fact, there are a number of medalists who have avoided the complications that usually accompany the disease. As Wesley notes, "I was at the medalist reunion and, while there are clearly people there who have complications, these people looked like any other bunch of old folks. So there's something that sets that group apart, and I think that's the real question. There must be something detectable or measurable that would explain the avoidance of complications in some people while other people have so many of them. We need to find out what it is."

The 50-year medalists represent a unique collection of individuals with long-standing type 1 diabetes who have mostly avoided the complications of the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), and heart that can accompany the disease. Individuals in this group may have certain

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factors—genetic, environmental, psychological, and physiological—that have contributed to their survival with long-term diabetes and protected them from the disease's complications. A group of investigators at Joslin agree that the medalists may hold keys to preventing the complications of diabetes. They invited the Joslin's 50-year medalists to participate in a clinical research study, the 50-Year Medalist Study, examining the outcomes of long-term type 1 diabetes and aiming to identify factor(s) that contribute to the longevity of this unique group and lead to resistance to complications.

Of his participation in the Joslin 50-Year Medalist Study, which seeks to identify factors that contribute to longevity and resistance to diabetes complications, Wesley says "I think that's the key to get the information about what to do [to combat type 1 diabetes]. It's the future."

Wesley didn't hesitate to participate in the study and feels that participation hasn't been too taxing. "It's not complicated," he responds when asked about his role in the study, "[I need to] be available to come back and get checked out occasionally." But, Wesley's participation goes well beyond that. He will make a special contribution to this research study some day in the future by donating some of his tissues post-mortem for analysis. Wesley feels that this contribution is critical: "I think that's the key to get the information about what to do [to combat type 1 diabetes]. It's the future," he explains, noting that type 1 diabetes is increasing in its frequency. By studying tissues like Wesley's, scientists hope to uncover new ways to prevent, reverse, and treat type 1 diabetes and its complications. "It's one thing I can do," shares Wesley, "If any part of my body would help understand that or identify [a factor/factors], I think that would be a great thing to have."

A Lifetime of Improvements in Diabetes Care

In his over 50 years with type 1 diabetes, Wesley has seen significant change in treatment and care of the disease. At the time he was diagnosed, people could only check sugar levels with urine tests, which recognized high but not dangerously low sugar levels and reflected past, not current, sugar levels; more reliable methods for testing sugar levels in the blood had not yet been developed. As Wesley explains, at that time, "you just tried to avoid the extremes, which in a way made sense because we had no way of checking blood sugars. I think everyone I knew who resorted to doing urine tests realized it was of no benefit to tell you what your blood sugar was at the time. You tended not to pay any attention to the urine test."

For Wesley the big step was when blood sugar testing arrived: "That is the big development as far as I'm concerned," he says, "because it allows control. I tell people that's the greatest gift that we've had." Today people with type 1 diabetes can wear continuous glucose monitors that provide real time information about a person's blood sugar levels and insulin pumps that can provide doses of insulin without injections. Artificial pancreas technologies that link these monitors and pumps and allow automatic release of insulin in response to high blood sugar readings are in clinical tests, and a device that suspends delivery of insulin when blood sugar levels drop dangerously low has already been approved by the U.S. Food and Drug Administration. In addition, certified diabetes educators have come on the scene to help with giving advice on the day-to-day management of diabetes.

Wesley has also loved physical activity and the outdoors, especially the mountains of Montana, and feels that exercise is vital to his health and managing his diabetes. "Daily exercise can be worked into any schedule," he shares, "everyone, especially those

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with diabetes, can find something they enjoy and can do almost every day." Wesley has found enjoyment in simple activities, like walking the family dog and gardening, and in more challenging ones, like scaling the highest peaks in Montana and Idaho. He and his wife also love to run and have participated in everything from local fun runs to marathons. Wesley even ran while he was practicing medicine, slipping it into his busy schedule—"It allowed me to burn up a lot of energy in a short time," he explains. Even still, Wesley is also quick to credit his good health to luck and his strong partner in the journey, his wife.

So, has diabetes slowed Wesley down at all? "I think it has taken the edge off things," he shares, "but if you are determined you can do things anyways. I think the main trouble is that you never can really forget about it. You can't just say I'm not going to take my insulin for a few days. Unfortunately, it's always there." But Wesley sees a lot of hope for people diagnosed with type 1 diabetes today in all the improvements in technology, changes in practice resulting from research results, and people available to help. The outlook is encouraging, especially with the promise the Joslin 50-Year Medalist Study holds to uncover protective factors for type 1 diabetes and its complications. He muses, "These days there are going to be far too many applicants for the 50-year award medals than Joslin is going to be able to give out because of the fact that people can now control their diabetes." That is cause for celebration.

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Paul and Tim Daly

Looking Out for One Another and Taking on Type 2 Diabetes

Paul Daly (left) and Tim Daly (right). ***Photo by Roberta Daly.***

Paul and Tim Daly have always been close. Identical twins from a close-knit family, they served in the Army together, attended the same technology school post-enlistment, and worked together, later, at Tim's video store in Framingham, Massachusetts. As they tell it, having a twin brother was "like being born with a best friend," and looking after one another was a lesson they learned early. "One of our uncles said 'look, you always back up your brother,'" Paul explains. "And that stayed with me. Whether you're with friends or wherever, you always back each other up. It's like the Golden Rule."

Decades later, it's a rule they still follow. Of course, while the rule may once have meant uniting to confront a schoolyard bully, today it more often means confronting diabetes.

In the mid-1990s, Paul's doctor told him he had elevated blood glucose that put him at risk for the disease. "The mistake I made was ignore them, 'cause I felt good," Paul says ruefully. He thought at the time that "risk for diabetes" meant "I wasn't sick, so I didn't need to worry about it." But about 6 months later, he found he was thirsty all the time. He learned from his doctor he had gone on to develop type 2 diabetes, and he knew he had to tell Tim as soon as possible, to make sure Tim knew "risk for diabetes" was something he *did* have to worry about.

When his doctor told him he was at risk for type 2 diabetes, "the mistake I made was ignore them, 'cause I felt good," Paul says ruefully. When he later developed the disease, he told his twin brother, Tim, as soon as possible, to make sure Tim knew "risk for diabetes" was something he did *have to worry about.*

After Paul relayed the news of his diagnosis, a family friend told Tim about a brochure she'd seen for a new study that was recruiting participants to see if type 2 diabetes could be prevented or delayed. Tim was struck by the timing, hearing about the study so soon after finding out about Paul's diabetes. "If this isn't a message from God…" he recalls thinking. He wasted no time before calling to set up an appointment with the staff of the just begun Diabetes Prevention Program (DPP). Through blood tests provided by the study, he found out he was in much the same place Paul had been 6 months before: he did not yet have diabetes, but he was dangerously close. The lesson of Paul's experience was not lost

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on Tim, who readily agreed to enroll, thus becoming one of the DPP's earliest participants.

The Diabetes Prevention Program

The DPP, led by NIDDK with additional support from other NIH Institutes and Centers, the Centers for Disease Control and Prevention, the Indian Health Service, and non-governmental organizations, recruited adult volunteers at 27 clinical centers around the United States. Participants were randomly divided into different treatment groups. The first group, called the lifestyle intervention group, received intensive training in diet, physical activity, and behavior modification. By eating less fat and fewer calories and doing moderate exercise, such as brisk walking, for a total of 150 minutes a week, they aimed to lose 7 percent of their body weight and maintain that loss. This intervention was based on extensive behavioral research that suggested it would be a sustainable approach to modest weight loss for a high proportion of participants. The second group took the generic diabetes drug metformin twice a day. The third, a control group, received placebo pills instead of metformin. The metformin and placebo groups also received information about diet and exercise, but no intensive behavior change counseling. All 3,234 study participants were overweight or obese; and, although they did not have diabetes by the criteria used at the time, their fasting glucose levels were elevated, and they had impaired glucose tolerance (meaning their blood sugar remained elevated longer than normal after drinking water that contained a set quantity of glucose.) Forty-five percent of the participants belonged to racial and ethnic groups at increased risk for developing diabetes.

The study was a tremendous success. In fact, researchers announced the initial findings of the DPP in 2001, a year earlier than scheduled, because the results were so striking. The lifestyle intervention reduced participants' risk of developing diabetes by 58 percent, and was effective for both men and women, for all race/ethnic groups in the study, and for participants of different ages. Participants taking metformin lowered their risk of developing diabetes by 31 percent.

"I started logging in everything I was eating, the exercise that I was doing," Tim says, and he added more exercise to his week and modified his diet. The attention to detail paid off.

Tim Daly's DPP Experience

Tim was randomly assigned to the DPP lifestyle arm. "I started logging in everything I was eating, the exercise that I was doing," Tim says. "I weighed 200 lbs, so my goal weight was 186." He already exercised about 2 hours per week, playing basketball with friends—exercise Paul had not been getting, which may have contributed to the earlier onset of his diabetes. So Tim only had to add 30 minutes of brisk walking to get to the goal of 150 minutes. The diet portion was a bit more of an adaptation, but "my goal was 42 grams of fat/day. So I was focused on that—not the carbohydrates, just the fat. We'd go in restaurants and I had certain meals I'd picked out—that's what I'm going to have when I go to that restaurant." The attention to detail paid off: within 6 months, he'd reached his goal weight—and he continued to be diabetes-free.

Was it tough? "I didn't feel it was that difficult," Tim recalls. But sometimes his weight began to drift back up, and when that happened, a study coordinator found a powerful way to motivate him. "She said, 'Tim—do you realize I have people in the study and they'll go, "Oh, what's the point? My family has diabetes, I have family history—I'm going to get it. What's the point?"' and she'll go, 'Time out. I have a guy in the study whose twin brother has been

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diagnosed with diabetes. And so he's been in the study since 1996, and he's not diagnosed as diabetic.'" Tim certainly got the message: "Oh, God," he thought, "I've got to do my best!"

The Diabetes Prevention Program Outcomes Study

At the completion of the DPP, no one knew how long the interventions' benefits would endure, since the results were based on just 3 years of data. Therefore, after a "bridge" period from January to July 2002, when all participants learned the results and were offered a multi-session program explaining how to make the lifestyle changes, the Diabetes Prevention Program Outcomes Study (DPPOS) began, with most of the DPP volunteers taking part. DPPOS participants who received metformin during the DPP continue to receive metformin. The lifestyle intervention has also continued, albeit somewhat less intensively, for participants from the lifestyle arm who agreed to stay in for the DPPOS. In addition, classes encouraging adoption of the lifestyle changes were also made available to DPPOS participants from the metformin and placebo arms, but attendance was optional.

"When my weight goes up, all the numbers go up… all the stuff you want to keep down."

Through analysis of data collected from participants over an average of 10 years after their initial enrollment in the DPP, researchers were able to conclude in 2010 that the lifestyle intervention reduced the rate of developing type 2 diabetes by 34 percent during this time, compared with placebo. Importantly, study participants who received the lifestyle interventions in the DPP and DPPOS also had fewer cardiovascular risk factors, including lower blood pressure and triglyceride levels, despite needing fewer drugs to control their heart disease risk. Over the same 10 years, treatment with metformin reduced the rate of developing diabetes by 18 percent compared with placebo. Through DPPOS research we also now know that the lifestyle and metformin interventions were highly cost-effective: by helping keep the participants healthier than they would have been otherwise, the interventions reduced the participants' other (non-study associated) health care costs.

Of exercise, Paul says, "Afterwards, always you have more energy; you just feel better mentally because you worked a little bit toward a goal." And like Tim, Paul has changed his diet for the better.

So thanks to Tim and the many other former DPP participants who agreed to continue participating in research through the DPPOS, study scientists have learned a great deal about the long-term outcomes of the DPP interventions. And sticking with the program has also had tremendous benefits for Tim personally. "I go in twice a year now, and… historically… they have all the numbers. It's incredible. When my weight goes up, all the numbers go up: blood sugar, cholesterol—all the stuff you want to keep down. It was definitely related to my weight." Confronting those numbers helped Tim stay on-task, and keep taking good care of himself.

Tim's healthful lifestyle greatly slowed the gradual rise in blood glucose levels most people experience as they age, but did not arrest that rise completely. Thus, as judged by one test, Tim's blood glucose control has recently reached the range considered indicative of diabetes, and by DPPOS protocols, Tim

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is considered to have developed type 2 diabetes. From Tim's perspective, even though the DPP lifestyle intervention did not permanently prevent diabetes, it did help him remain free of this disease—and free of the challenges of managing it and related health complications—for 17 years after Paul's diagnosis. "With Tim jumping on it as soon as he found out—that made the difference," says Paul. Tim agrees: "It did!"

Further, the study—and the journey that began with Paul's diagnosis—imbued both men with the determination to remain as healthy and active as possible. Tim still exercises avidly. He plays golf with his buddies, and he doesn't use a cart. He and his wife also stay fit and active though a shared love of country line dancing. In addition, he recently started a new fitness program with his wife and one of his daughters, and reached his lowest weight in decades.

Paul, too, recognized the importance of adopting a healthier lifestyle. For fitness, he enjoys bicycling and walking on the beach. Both brothers have become great proponents of exercise. As Paul puts it, "Afterwards, always you have more energy; you just feel better mentally because you worked a little bit toward a goal." And like Tim, Paul has changed his diet for the better, usually opting for fish over red meat, for example. "Everything in moderation," he says. "And there are certain things to stay away from, too." While he acknowledges it can sometimes be challenging to forego certain foods, often he finds that it isn't. "I haven't eaten mayonnaise in like 20 years, now. I like mustard on everything, now, which I didn't before.... I like it better that way."

Leading by Example

The Daly brothers are committed to helping others learn that they, too, can change their lives for the healthier—and happier. They've been interviewed for National Public Radio,[1] and were featured in a segment of the HBO Documentary Series *The Weight of the Nation*.[2] But just as the story began with brothers, it remains for them about family. Explaining that his mother-in-law also has diabetes, he says he told his daughters, "heads up: this is what you got for genetics." "It's the same for my daughters," says Paul. "My wife has prediabetes [elevated blood glucose or insulin-resistance putting her at-risk for developing type 2 diabetes]; her father was diabetic; and my daughters see what I go through. So they're starting to adjust."

And of the family friend who told him about the DPP Tim says, "Every time I see her, I thank her."

Fortunately, for their families, Tim and Paul lead by example, looking out for each other, taking care of themselves, and making changes to lead longer, healthier lives. "We have to," Tim says reflectively. "You know, we want to enjoy our grandchildren, God willing." And of the family friend who told him about the study Tim says, "Every time I see her, I thank her."

[1] *http://www.npr.org/templates/story/story.php?storyId=122104219*

[2] *http://theweightofthenation.hbo.com/watch/main-films/Choices*

Early intervention to prevent obesity has the potential to improve health and reduce health care costs associated with obesity-related diseases now and in the future. In fall of 2013, the NIDDK and other Institutes sponsored a workshop on the Prevention of Obesity in Infancy and Early Childhood. This workshop brought together a diverse group of scientists to discuss what is known about obesity in children and infants and how childhood obesity might best be treated or prevented. This workshop aimed to provide the scientific background for future NIH supported research on interventions to prevent inappropriate weight gain during infancy and early childhood.

Obesity

Obesity has risen to epidemic levels in the United States. Individuals who are obese may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission.

More than one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Nearly 17 percent of children and teens ages 2 through 19 are also obese, and thus at increased risk for developing serious diseases both during their youth and later in adulthood.[3] Obesity disproportionately affects people from certain racial and ethnic groups and those who are socioeconomically disadvantaged.

The high prevalence of obesity in the United States is thought to result from the interaction of genetic susceptibility with behaviors and factors in the environment that promote increased caloric intake and sedentary lifestyles. Diet, activity, and aspects of our environment may also modify biologic factors in ways that promote obesity. Research is providing the foundation for actions to address this major public health problem by illuminating the causes and consequences of obesity, evaluating potential prevention and treatment strategies, and providing an evidence base to inform policy decisions. The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental approaches in families, schools, health care settings and other community settings; medical and surgical interventions; and combinations of these strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches. To help bring research results to those affected by obesity and their families, health professionals, and the general public, the Institute also sponsors health information programs.

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the Directors of the National Heart, Lung, and Blood Institute and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. These represent examples of NIDDK's broad spectrum of research efforts toward reducing the burden of obesity so that people can look forward to healthier lives.

BARIATRIC SURGERY AND WEIGHT LOSS

Surgically Remodeling the Intestines—and the Bacteria Within—To Treat Obesity: Gaining new insight into Roux-en-Y gastric bypass surgery, a treatment for obesity, researchers studying a mouse

[1] *Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm*

[2] *Ogden CL, et al. NCHS data brief, no 131. National Center for Health Statistics. 2013.*

[3] *Ogden CL, et al: JAMA 307: 483-490, 2012. For children and adolescents, obesity refers to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).*

model found that restructuring of the digestive tract leads to weight loss and metabolic benefits in part by altering the communities of bacteria that normally live in the intestines. Gastric bypass, a form of bariatric surgery, is thought to work in a number of ways, but the mechanisms are not well understood. Among various changes observed after gastric bypass are alterations in gut bacteria.

Thus, a team of researchers sought to determine whether the changes in types of bacteria (gut microbes) in the intestines after gastric bypass contribute to weight loss. To do this, they started with groups of obese mice that had been fed a high-fat diet, performed gastric bypass surgery on some of the mice and sham surgery (as a control) on others, and then analyzed intestinal bacteria. Despite access to unlimited food after surgery, the mice that had gastric bypass lost 29 percent of their initial body weight and maintained their lower weight throughout the study. These mice also had reduced visceral body fat, the type of fat most associated with metabolic disease, and other metabolic benefits. Their resident gut microbes changed rapidly after surgery, as different types of bacteria flourished in the newly renovated intestinal habitat. By contrast, a group of mice that had only sham surgery, which did not alter the intestinal tract, regained all of their initial body weight after recovering from surgery. These mice also had more body fat, and their gut bacteria differed from those of the gastric bypass group. The researchers then tested whether putting mice on a low-calorie diet would lead to similar changes in gut bacteria. Dieting did not have quite the same effect: the relative abundances of various types of bacteria from the mice on a diet differed from those of the gastric bypass group. Finally, the researchers investigated whether the unique collection of gut microbes resulting from gastric bypass could cause weight loss—even without gastric bypass surgery. They extracted bacteria from mice that had undergone gastric bypass and administered the bacteria to other mice that had not been treated surgically. Although the recipient mice did not lose as much weight as the gastric bypass group, the bacterial transfer did lead to some weight loss, along with reduced body fat.

This study indicates that changes in gut bacteria contribute to the weight loss and reduced body fat from gastric bypass surgery in mice. Because changes in gut bacteria have also been observed in people after surgery, this study also has implications for understanding how gastric bypass works in humans. Further research into the effects of gut microbes on weight and metabolism after gastric bypass in people may lead to interventions that achieve similar health benefits without the cost and risks of surgery.

Liou AP, Paziuk M, Luevano JM Jr, Machineni S, Turnbaugh PJ, and Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 5: 178ra41, 2013.

EFFECTS OF DIET AND EXERCISE

Health Effects of Diet and Exercise for Adults with Type 2 Diabetes and Obesity: Researchers in the Look AHEAD (Action for Health in Diabetes) clinical trial found that an intensive lifestyle intervention for weight loss was not sufficient to stave off heart disease and stroke in overweight and obese adults with type 2 diabetes, but it did yield a number of other health benefits.

Because type 2 diabetes is a devastating disease, increasing risk for cardiovascular disease and other complications, and because obesity also heightens risk for many diseases and disorders, researchers have been exploring ways to improve health for individuals with these conditions. Based on prior, short-term studies, weight loss has been recommended for overweight and obese patients with type 2 diabetes, but the effects on cardiovascular disease (CVD) over the long term had not been known. To conduct the Look AHEAD clinical trial, investigators at 16 centers around the United States recruited 5,145 volunteers. The participants were women and men of diverse racial/ethnic backgrounds, ages 45 to 75 at the start of the study, who had type 2 diabetes and were overweight or obese. The researchers designed a lifestyle intervention that aimed for a seven percent weight loss through decreased calorie intake, and in particular, reduced calories from dietary fat, along with 175 minutes of moderate intensity physical activity per

week. The intervention included regular group and individual counseling sessions, liquid meal replacement products for some meals, and a toolbox of additional strategies to assist those having difficulty with weight loss. Half of the participants were randomly assigned to receive this lifestyle intervention. For comparison, the other half of the participants were assigned to a much less intensive program of diabetes support and education, comprised of several group sessions focused on diet, exercise, and social support. For all participants, the investigators recorded CVD events: instances of death from any cardiovascular cause, non-fatal heart attack or stroke, and hospitalization for a condition called angina. They also monitored other health conditions.

After nearly 10 years of follow up, the researchers found that the numbers of CVD events were not significantly different between the intensive lifestyle intervention group and the diabetes support and education group. This similar number of CVD events was observed even though there were improvements in a number of disease risk factors in the lifestyle intervention group, with less use of medication. These benefits included greater weight loss; improved blood pressure; increased fitness, as measured by an exercise test; and improved blood glucose (sugar) control.

Additional analyses of data from the first several years of the Look AHEAD trial have identified many other health benefits of the lifestyle intervention. For example, the researchers investigated whether the intervention led to partial or complete remission of type 2 diabetes over the first four years of the study, and, as reported recently, they found that it did, although at modest rates. After one year, about 11.5 percent of those in the intervention group experienced at least partial remission of their diabetes, as defined by blood glucose levels decreasing to prediabetes or normal levels without need for diabetes medications. By the fourth year of the study, 7.3 percent of participants in the lifestyle intervention had at least partial remission of their diabetes. Those in the control group experienced a smaller remission rate of only two percent at both time points. Remission in the intensive lifestyle intervention group was more likely among those who had lived with diabetes for less than two years at the study's outset, and who had relatively low (although still diabetic) glucose levels, did not yet need insulin therapy for their diabetes, and had greater weight loss and fitness improvements during the study. The association between remission and shorter duration of type 2 diabetes suggests that starting healthy lifestyle changes early in the course of the disease may lead to better outcomes.

In another recently reported analysis, researchers analyzing the first four years of Look AHEAD data found that the lifestyle intervention reduced levels of obstructive sleep apnea, a serious breathing disorder during sleep that is associated with obesity. Among other benefits, researchers have previously reported a decrease in urinary incontinence, improvements in quality of life, and less of a decline in physical mobility as a result of the lifestyle intervention. At this point, because there was not a reduction in CVD events, the intervention phase of Look AHEAD has concluded. However, researchers are continuing follow up of the study participants to evaluate longer-term effects. This research will help inform decisions about management of type 2 diabetes, to help people improve their health and quality of life.

Look AHEAD Research Group, Wing RR, Bolin P, et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. N Engl J Med 369: 145-154, 2013

Gregg EW, Chen H, Wagenknecht LE, et al. Association of an intensive lifestyle intervention with remission of type 2 diabetes. JAMA 308: 2489-2496, 2012.

"Maintain, Don't Gain"—A Weight Management Approach To Help Those Already Overweight or Obese: Researchers have achieved promising results in an intervention to help overweight and obese African American women prevent further weight gain, using a combination of primary care and community settings, along with technology accessible to those who are socioeconomically disadvantaged. The intervention aimed not for weight loss, but rather to improve the overall well-being of the participants and maintain their current body shape—a message that they hoped would resonate with the participants. By helping overweight

and moderately obese individuals avoid further weight gain, the researchers hoped to prevent the additional health risks associated with extreme obesity. The researchers recruited women from primary care centers in a socioeconomically disadvantaged area in the South. Participants were randomly assigned either to the intervention or, for comparison, to their usual care. For 12 months, the women in the intervention group received tailored behavior change goals (for example, no sugar-sweetened beverages), weekly self-monitoring with computer-generated telephone calls, monthly counseling calls from a registered dietician, skills training materials, and a one-year YMCA membership.

Measuring weight at the end of the 12 month intervention, and then again at 18 months, the researchers found that the women in the intervention group better maintained their initial weight: 53 percent had weights at or even below their weight at the start of the study, compared to 39 percent of those in usual care. On average, the women in the intervention group had a slight weight loss of approximately two pounds, while those in the usual care group on average gained that amount of weight. Many of the women had other health risk factors at the outset of the study, but the intervention did not affect blood pressure, blood glucose, or several other cardiovascular risk factors. Importantly, although recruiting from a primary care system, the intervention was largely delivered in the community with dieticians and relatively inexpensive electronic health technologies. This strategy circumvents issues related to insufficient reimbursement, time, and training that may hinder effective weight management solely by primary care physicians. This strategy could also be implemented broadly in communities disproportionately affected by obesity. Longer-term studies may help researchers determine whether—by fending off the usual weight gain over time—this intervention could reduce type 2 diabetes and other obesity-associated diseases.

Bennett GG, Foley P, Levine E, et al. Behavioral treatment for weight gain prevention among black women in primary care practice: a randomized clinical trial. JAMA Intern Med 173: 1770-1777, 2013.

BODY WEIGHT AND THE BRAIN

Let's Not Sugar Coat It: Evidence Is Emerging that Fructose and Glucose Elicit Different Brain Responses: Researchers have found that in humans, ingestion of glucose, but not fructose, activates brain pathways and stimulates hormones that promote feelings of satiety, fullness, and reward. Consumption of the simple sugar fructose in the United States has risen significantly over the past few decades, concomitant with an increase in the prevalence of obesity. Several studies have provided evidence suggesting that fructose consumption does not have the same effects as glucose on circulating hormones that modulate hunger and satiety. While research using animal models has demonstrated that fructose affects regions of the brain (*e.g.*, the hypothalamus) that help regulate feeding behavior, corresponding analyses in humans have been technologically challenging to perform. Scientists have now used a new method of determining the effects of fructose or glucose on specific regions of the human brain by using functional magnetic resonance imaging (fMRI) to quantify cerebral blood flow—a proxy measure for brain activity. Twenty normal-weight participants, after fasting, drank beverages containing either glucose or fructose; the resulting effects on the brain were analyzed, along with circulating hormones (from blood samples), and other changes. Glucose consumption led to reduced blood flow in the hypothalamus and other regions of the brain associated with hunger, changes which were not seen with fructose consumption. Glucose ingestion led to a significantly greater induction of circulating insulin and GLP-1, two hormones that promote satiety, than did fructose consumption. Although both sugars induced functional connectivity—a statistical measurement of the communication between parts of the brain—between the hypothalamus and various other brain regions, only glucose consumption induced functional connectivity to the striatum, a reward center of the brain. Participants who drank the glucose beverage reported feeling full and satiated, but those who drank the fructose beverage did not.

Together, these data support the idea that in people, glucose, but not fructose, promotes satiety, in part because of its effects on the activity of specific regions of the brain, which differ from those of fructose. This study was limited by several potential issues, including technical constraints, the small number of participants, and the complicating fact that the study design does not reflect real-world conditions (*e.g.*, people typically consume sugars and other nutrients in combination, not individually). Although the role of fructose's effects on the brain in the development of obesity was not directly explored, these results suggest that previous findings in animal models are relevant to humans, with respect to differences between fructose and glucose. Future research could shed light on how these effects may relate to food consumption and health.

Page KA, Chan O, Arora J, et al. Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA 309: 63-70, 2013.

Discovery of Body Weight Regulation by the Protein MRAP2: Researchers discovered that the MRAP2 protein regulates growth and body weight in animals, and MRAP2 deficiency may contribute to severe obesity in humans. Two research teams studied MRAP2 because its suspected biological partner, a protein called MC4R, is known to be critical in metabolism; people born with genetic deficiencies in MC4R develop severe obesity as children. To investigate MRAP2 further, one team generated mice with a genetic mutation that caused a deficiency of this protein. Without MRAP2, the mice became extremely obese, even when their food was limited so they could eat only as much as normal mice. The MRAP2-deficient mice only attained a normal weight when the researchers further restricted their allotted food to less than what a typical mouse would eat—a finding that may help explain why weight control through dieting is a constant challenge for people with genetic predisposition to obesity.

Because MRAP2-deficient mice fed normal portions still became obese, the researchers realized that MRAP2 may affect body weight in ways other than through appetite and food intake. They thus examined whether MRAP2 influences how much the mice move, their calorie burning, or body temperature, but found no differences between mice with and without MRAP2. Although these results alone do not clarify the mechanism by which MRAP2 functions, additional studies by both teams, using mice and another experimental organism, zebrafish, revealed several clues. MRAP2, like MC4R, works in the brain; MRAP2 and MC4R physically interact; and MRAP2 influences MC4R's activity. Finally, one of the research teams analyzed genomic data from people whose obesity began in childhood, and found that a few of these individuals had rare genetic variants that may impair MRAP2. The variants were not seen in non-obese people.

This research sheds light on a potential rare genetic form of obesity and yields insights that may lead to the design of new therapeutics that target MRAP2.

Asai M, Ramachandrappa S, Joachim M, et al. Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity. Science 341: 275-278, 2013.

Sebag JA, Zhang C, Hinkle PM, Bradshaw AM, and Cone RD. Developmental control of the melanocortin-4 receptor by MRAP2 proteins in zebrafish. Science 341: 278-281, 2013.

Researchers Identify Malfunctioning Satiety Signal in Mice Fed a High-fat Diet and Its Link to Dopamine in the Brain: A group of scientists has identified a chemical produced in the gut that regulates the sense of satiety, or fullness, in mice after dietary fat consumption by inducing dopamine release in the brain, a process that is disrupted after excessive high-fat feeding. The feeling of satisfaction following ingestion of food is caused by a chemical in the brain called dopamine. A significant amount of dopamine is usually released by the brain after eating, resulting in a sense of gratification or reward. For unknown reasons, however, prolonged eating of a high-fat diet leads to desensitization of this signal,

which is believed to contribute to overeating in order to compensate for a dampened feeling of satisfaction.

Researchers studying this phenomenon in a mouse model identified a chemical produced by the gut in response to eating that can cause dopamine release in the brain. However, if the mice are fed high-fat diets for several weeks, the levels of this chemical, called oleoylethanolamine (OEA), are reduced. When the group supplied OEA directly to the intestine, the mice reduced their fat intake and lost weight, supporting OEA's role in promoting satiety. The scientists, interested in how OEA was signaling to the brain in these mice, found that OEA's effects require a factor called PPARα. They also found that the signal generated by OEA and PPARα is probably transmitted through the vagus nerve, which carries signals between the intestines and the brain, because infusion of OEA directly into the stomach did not seem to affect mice that lacked functional vagus nerves. The scientists next enabled the mice to self-administer fat directly to their stomachs, bypassing the mouth and therefore the ability to taste the incoming food. When mice on prolonged high-fat diets were fed in this manner, they were less likely to keep self-administering the fat than the mice on low-fat diets, suggesting that the dopamine-mediated reward response was impaired in the high-fat fed mice. But when mice on the high-fat diet were given OEA, they self-administered more fat, suggesting that OEA restores the dopamine-mediated reward signal associated with eating that had weakened in these mice.

This study supports a model whereby a long-term, high-fat diet suppresses the production of OEA in the gut, resulting in a decrease in the production of dopamine in the brain and a dampening of the reward signals that usually accompany eating foods high in fat. This could mean that the desire to eat high-fat foods is strongly affected by these chemical signals originating in the gut, which could point to new therapeutic options for suppressing appetite and treating obesity in people.

Tellez LA, Medina S, Han W, et al. A gut lipid messenger links excess dietary fat to dopamine deficiency. Science 341: 800-802, 2013.

GENETICS OF OBESITY

Gene Linked to Obesity Has Role in Different Types of Human Behavior: Researchers discovered that a gene with a known role in obesity also plays an unexpected role in human behavior. The gene, called *SH2B1*, is a key regulator of leptin sensitivity in mice; leptin is a hormone that suppresses appetite. Previous research showed that mice lacking the *SH2B1* gene have impaired leptin and insulin signaling, making them severely obese and insulin resistant. To investigate the role of *SH2B1* in human metabolism, scientists examined whether obese people had mutations in their *SH2B1* gene. They studied people with severe early-onset obesity (developed before age 10) who also had higher than expected levels of insulin resistance for their weight. They identified four different types of mutations in the *SH2B1* gene that were not found in people who are normal weight. The mutations were inherited from their overweight or obese parents. The people with these mutations had excessive appetites and at adulthood were a shorter height than average. A surprising finding was that they also had behavioral abnormalities, as reported by their families and health care providers. These abnormalities included delayed speech and language development, aggressive behavior, and a tendency toward social isolation. Further experiments showed that some of the identified mutations impaired leptin signaling while others did not, suggesting that the SH2B1 protein may be exerting some of its effects through cellular pathways that are independent of leptin. This research sheds new light on the human *SH2B1* gene and suggests that it not only plays a role in obesity, but may also be an important factor in controlling certain types of human behavior.

Doche ME, Bochukova EG, Su HW, et al. Human SH2B1 mutations are associated with maladaptive behaviors and obesity. J Clin Invest 122: 4732-4736, 2012.

PREDICTING BODY WEIGHT AND EFFECTS OF DIET AND ACTIVITY WITH MATHEMATICAL MODELING

"The Biggest Loser" Study Predicts Sustained Weight Loss Through Modest Changes in Diet and Exercise: With a validated computational model for predicting weight loss, a research study has quantified the dramatic diet and exercise intervention strategy employed in "The Biggest Loser" television program. It found that this intervention would not be sustainable over time, but a more modest and feasible diet and exercise could maintain the weight loss. "The Biggest Loser" program, although highly successful, has been criticized for portraying an extreme diet and exercise intervention regimen that could raise unrealistic expectations for weight loss. The program shows obese adults losing large amounts of weight over several months, initially isolated on a ranch, followed by an extended period at home.

Scientists took advantage of this cost-efficient opportunity to study the television program's 16 obese contestants already engaged in an intensive lifestyle intervention. As part of the program, researchers measured body fat, total energy expenditure and resting metabolic rate—the energy burned during inactivity—three times: at the start of the program, at week 6, and at week 30, which was at least 17 weeks after participants returned home. Participation in the program led to an average weight loss of 128 pounds, with about 82 percent of that coming from body fat, and the rest from lean tissue like muscle. Preserving lean tissue, even during rapid and substantial weight loss, helps maintain strength and mobility and reduces risk of injury, among other benefits.

A scientist at NIDDK then used a mathematical computer model of human metabolism to calculate the diet and exercise changes underlying the observed body weight loss. Because the TV program was not designed to directly address how the exercise and diet interventions each contributed to the weight loss, the computer model simulated the results of diet alone and exercise alone to estimate their relative contributions. At the competition's end, diet alone was calculated to be responsible for more weight loss than exercise, with 65 percent of the weight loss consisting of body fat and 35 percent consisting of lean mass like muscle. In contrast, the model calculated that exercise alone resulted in participants losing only fat, and no muscle. The simulation of exercise alone also estimated a small increase in lean mass despite overall weight loss. In addition, the simulations suggest that the participants could sustain their weight loss and avoid weight regain by adopting more moderate lifestyle changes—like 20 minutes of daily vigorous exercise and a 20 percent calorie restriction—than those demonstrated on the television program.

These findings suggest that a more moderate weight loss strategy, rather than the extreme lifestyle intervention depicted in "The Biggest Loser," would be preferable for sustained weight loss in many people who are obese.

Hall KD. Diet versus exercise in "The Biggest Loser" weight loss competition. Obesity (Silver Spring) 21: 957-959, 2013.

Mathematical Model Predicts Effects of Diet and Physical Activity on Childhood Weight: Scientists have created and confirmed the accuracy of a mathematical model that predicts how weight and body fat in children respond to adjustments in diet and physical activity. Excess weight in children can lead to lifelong health problems, such as type 2 diabetes and high blood pressure. However, the amount of energy (calorie) intake that leads to excess weight and development of obesity in growing children has been difficult to quantify. The challenge of taking into consideration healthy weight gain from normal growth has impeded the development of accurate mathematical models to simulate and predict body weight changes in children and adolescents.

NIDDK researchers have now modified a mathematical model, originally designed for and validated in adults, to account for children's unique physiology, including changes in body composition (the amounts of body fat and other body tissues) as they grow. The researchers

analyzed data from children ages 5 to 18 years to create the model, and tested it by comparing predictions to actual changes in children as measured in clinical studies that were not used to build the model. The model accurately simulated observed changes in body composition, energy expenditure, and weight. Model simulations also suggest that obese children may be eating far more calories for each pound gained, compared to adults. For example, children under age 10 were predicted to require more than twice the calories per pound of extra weight than an adult would need to gain a pound. Additionally, the model suggests that there may be therapeutic windows of weight management when children can "outgrow" obesity without requiring weight loss, especially during periods of high growth potential in males who are not severely obese at the onset of treatment. While the model may help to set realistic expectations, a controlled clinical trial will be needed to determine if it is an effective tool for weight management.

Hall KD, Butte NF, Swinburn BA, and Chow CC. Dynamics of childhood growth and obesity: development and validation of a quantitative mathematical model. Lancet Diabetes Endocrinol 1: 97-105, 2013

Turning Up the Heat—Important New Insights into the Biology of Brown Fat

New discoveries about the properties of brown and beige fat could inform research to develop potential new approaches for reducing obesity and its associated diseases.

Mammals harbor different kinds of adipose (fat) tissue in various regions of the body. Calorie-storing white adipose tissue (WAT) is the most abundant, and can be found surrounding internal organs, where it is referred to as visceral or abdominal fat, and just under the skin, a location termed subcutaneous. In contrast to the fat-storing WAT, brown adipose tissue (BAT) burns calories to generate heat. The heat-generating activity of brown fat is induced by cold weather, and contributes to a phenomenon known as "non-shivering thermogenesis" in which heat is produced as a by-product of specific biochemical processes that aid mammals in staying warm during hibernation.

Recent research has identified a second type of inducible brown fat cell (alternately called beige, brite (for brown in white), or recruitable BAT cells) that exhibits some of the characteristics of classic brown fat. The beige fat cells appear within portions of white fat and muscle tissue, in response to cold or other nervous system triggers. Because of the calorie-burning capability of both types of brown fat, many scientists believe that BAT could serve as an ideal target for the development of treatment strategies for obesity in humans. However, while rodents maintain patches of brown fat near the neck throughout life, classic brown fat largely disappears after infancy in humans. Interest in the therapeutic potential of BAT has been rekindled with the discovery of alternative inducible forms of BAT present in adult humans. New reports shed important new light on the anatomy, physiology, and molecular properties of human and rodent fat tissue.

In one recent study, while investigating the molecular control of brown fat activity, researchers uncovered a novel way that mammals regulate body heat—by promoting beige fat production when classic brown fat mass is reduced. Scientists genetically modified mice to lack the protein BMPR1A in a region of the developing embryo from which the bulk of BAT arises. Removing BMPR1A in these cells dramatically reduced the size of many BAT patches (also known as BAT depots). Unexpectedly, the body temperatures of adult mice lacking BMPR1A were no different from their normal littermates. Furthermore, after prolonged exposure to cold temperatures, body temperatures initially dropped in normal and genetically modified mice, but subsequently came back to normal, presumably due to brown fat activation. While the normal mice recovered in 48 hours, the mice lacking BMPR1A also recovered but required 11 days to return to normal. This surprising result suggested that cold-inducible beige fat cells might be recruited to compensate for the loss of other BAT. Further analysis showed that beige fat was indeed induced in WAT. The researchers confirmed this finding with other mouse models in which BAT activity was disrupted. This study identified a novel and complex interplay between brown and beige fat, and suggests that this compensatory relationship must be taken into account when designing brown fat-targeted therapeutics for altering energy balance.

In another study, researchers sought to better understand if beige fat cells can respond to cold exposure directly, or, as has been previously thought, only to cold-generated signals sent from the nervous system and interpreted by WAT. The scientists used mice genetically modified to lack key proteins, called β-adrenergic receptors, that interpret signals sent from the brain in response to cold temperature. These mice were then exposed to moderately cold conditions, and different fat depots were isolated to see whether genes involved in heat production (thermogenesis) were turned on. In beige fat cells without β-adrenergic receptors, thermogenic genes were turned on, but at

levels much lower than normal. Surprisingly, these genes were also turned on to a similar extent in subcutaneous WAT with and without the β-adrenergic receptors. To investigate these effects in the absence of influence from the nervous system and brain, the researchers grew isolated white fat and beige fat cells in the laboratory, exposed them to cold temperature, and found that even in isolation, specific thermogenic genes were turned on, and could be turned off again by returning the cells to normal temperature. Cellular respiration—one measure of a cell's energy output—also increased with cold exposure. Isolated classic brown fat cells did not exhibit the same responses to direct cold exposure, suggesting that their activity is induced by indirect factors (*i.e.*, the nervous system). While most of these experiments were done in mice or with mouse cells, the researchers also tested isolated human cells from subcutaneous fat, and found that these cells similarly turned on thermogenic genes in response to cold temperature. Together, these results reveal a previously unknown direct response to cold temperature by white and beige fat cells. Scientists will now explore the mechanism of the direct response to determine whether this system can be exploited to develop weight loss strategies.

Studies in mice have greatly illuminated the basic biology of fat tissue and how the body coordinates the thermogenic response to cold temperature. The applicability of findings from rodent models to human biology, however, is always a concern, and technical limitations have impeded progress in identifying and characterizing human BAT.

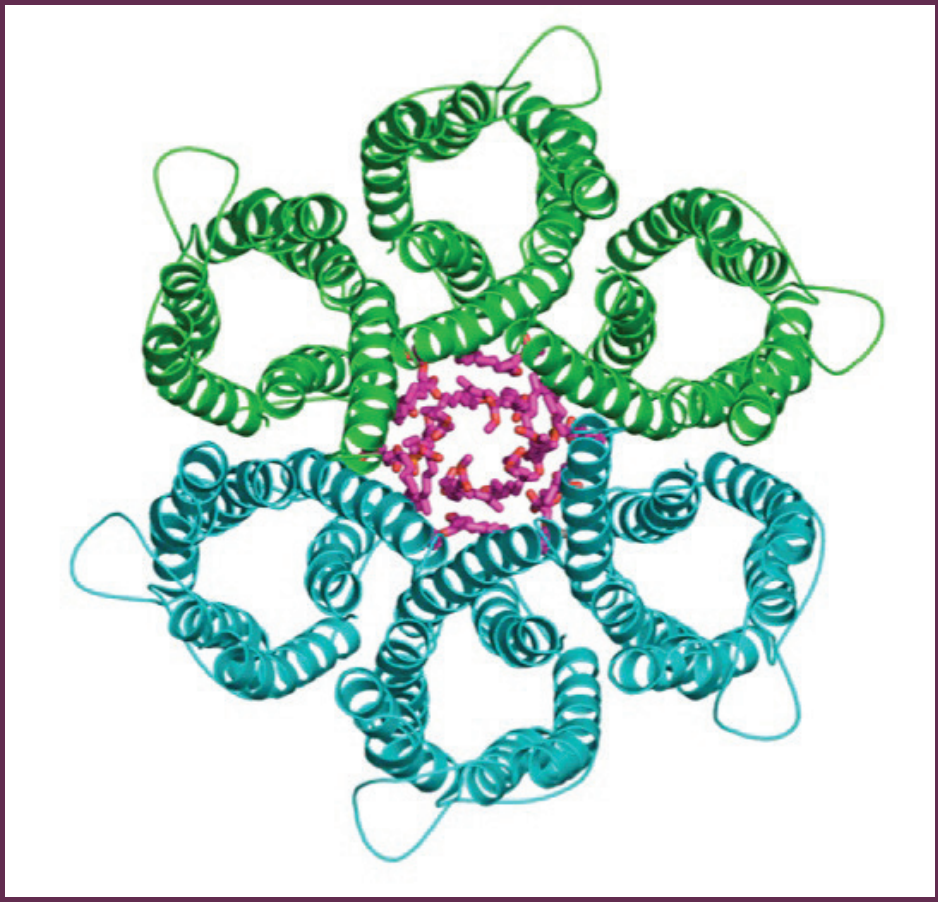
To begin to address this issue, a third study characterized the complex anatomy and molecular nature of fat tissue derived from the human neck. Scientists isolated samples of fat tissue from the necks of 31 participants who had given consent and were already undergoing surgery for other reasons. The researchers then carefully examined five different depots at progressively deeper positions within the neck. Molecular and cellular analyses revealed that broadly, the fat tissue closer to the surface exhibited properties similar to mouse WAT, whereas the deeper fat depots more closely resembled mouse BAT. Importantly, however, there was considerable variability between individuals. The researchers isolated a population of cells and, using specific conditions, induced them to become brown fat cells. These brown fat cells could indeed turn on thermogenic genes in response to a chemical that "tricks" the cells into thinking that the body was exposed to cold temperatures. Moreover, cells from the deeper fat tissues of two individuals had the capacity to burn about 100 times more calories—as determined by oxygen consumption rate—than subcutaneous fat tissue, and about half as much as mouse BAT. Together, these results suggest that adipose depots deep in the human neck most closely resemble rodent BAT in anatomy and function.

These reports greatly advance the current understanding of brown, beige, and white adipose tissues, describing novel properties and complex relationships within the heat regulation system. The detailed anatomical, molecular, and functional characterization of human neck adipose tissue will aid in the translation of findings in rodents to human biology, and provides critical new information that can potentially be used to develop therapeutic strategies to alter energy balance in people.

Cypess AM, White AP, Vernochet C, et al. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med *19: 635-639, 2013.*

Schulz TJ, Huang P, Huang TL, et al. Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. Nature *495: 379-383, 2013.*

Ye L, Wu J, Cohen, P, et al. Fat cells directly sense temperature to activate thermogenesis. Proc Natl Acad Sci USA *110: 12480-12485, 2013.*



Helicobacter pylori (*H. pylori*) is a type of bacteria that causes stomach ulcers and can also cause stomach cancer. Shown here is the structure of the urea channel of *H. pylori*, which is essential for the bacterium to survive in the acidic stomach environment. The complex is made up of six subunits (in green and blue), making up six individual urea channels surrounding a lipid core (in purple). Having detailed information about essential structures of pathogens like *H. pylori* can aid in identifying targets for new treatments.

Image courtesy of Dr. Hartmut Luecke, University of California, Irvine and Dr. George Sachs, University of California, Los Angeles. Adapted by permission from Macmillan Publishers Ltd: Nature, Strugatsky, et al. Structure of the proton-gated urea channel from the gastric pathogen Helicobacter pylori*. 493: 255-258, copyright 2013.*

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, the latest date for which reliable statistics are available, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare, collectively, they exact a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the United States and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract, leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori*, or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, which is one of the cancer types still on the rise in the United States. Gastroparesis

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

is another functional bowel disorder, which is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers to addressing fecal incontinence, and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbe community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop, and how they can best be treated.

NUTRITION AND DISEASE

Connecting Food, Genes, and Rare Metabolic Diseases: Researchers have mapped out an intricate network of genes that connect metabolism, responses to different foods, and rare metabolic diseases in a study of tiny worms. Seeking to understand the effects of different diets on gene activity, the research team selected a model organism that would enable them to carry out experiments not possible in humans. With a miniature digestive system, a transparent body, and a genome readily amenable to manipulation, the small (one millimeter) worm, *C. elegans*, affords numerous advantages for discovering genes that can then be analyzed in people. Worms will also eat different types of food in the laboratory, allowing examination of responses to different diets.

As a starting point for their study, the researchers found a gene with activity that ramped up or diminished depending upon what the worms ate; this gene appears to be involved in breaking down certain amino acids, which are components of proteins. They attached this gene to a fluorescent marker that, reflecting the gene's activity, would glow to a greater or lesser extent based on the type of food eaten. Using fluorescence as a dietary sensor (visible through the transparent worms), along with other experimental techniques, the researchers were able to identify over 180 other genes that worked with the first one in a vast network to respond to dietary nutrients and other aspects of metabolism. Some of these genes encode enzymes important in breaking down various components of food, and some of the genes appear regulatory in nature, modulating the extent to which other genes are activated or shut down. Applying their new knowledge to gain insight into human metabolism, the researchers found that many of the worm genes had counterparts in humans. This network may thus mirror similar interconnections among human genes, elucidating pathways by which the body senses different dietary nutrients or other metabolic signals, and modulates gene activity to be able to digest and process the nutrients or respond in other ways as needed. Moreover, several of these human genes are known to be associated with rare diseases. Collectively referred to as inborn diseases of amino acid metabolism, these diseases are caused by mutations in the genes and are marked by incomplete breakdown of certain amino acids; treatments involve specific dietary modifications. Thus, in addition to advancing understanding of metabolism and response to diet, this study may also lead to new avenues for therapeutic development to improve the health of people with inborn metabolic diseases.

Watson E, MacNeil LT, Arda HE, Zhu LJ, and Walhout AJM. Integration of metabolic and gene regulatory networks modulates the C. elegans *dietary response. Cell 153:253-266, 2013.*

GUT MICROBES IN HEALTH AND DISEASE

Gut Microbes Contribute to Severe Malnutrition in Young Malawian Twins: A study of young twin pairs in Malawi has shown that gut microbes may play an important role in causing severe malnutrition in children that persists in spite of nutritional interventions. Malnutrition is the top cause of child mortality in the world. Childhood malnutrition is common in some African countries like Malawi, where the diet is deficient in protein and the rate of infant mortality is one of the highest in the world. A type of severe malnutrition called kwashiorkor is thought to result from a combination of inadequate nutrient intake, particularly of protein, and other environmental factors. Although it is not fully understood why some children develop kwashiorkor, evidence from past studies has indicated a possible role in early development for the trillions of microbes that reside in the human gut. A group of researchers from the United States, Malawi, and the U.K. conducted a study of identical and fraternal twins born in Malawi to identify any links between gut microbes and severe malnutrition. They collected fecal samples every two weeks from pairs of twins with malnutrition under three years of age—before, during, and after they were given the standard treatment of ready-to-use therapeutic foods or supplements. In some cases, both twins became well-nourished; in others, one or both of the twins relapsed and again became malnourished after the nutritional therapy. The scientists concentrated on those pairs—similar in genetics, diet, and environment—in which one twin was well-nourished after treatment, while the other developed kwashiorkor. They analyzed the fecal samples to detect DNA from gut microbes, and found that different gut microbes were present in the well-nourished compared to the malnourished twin.

To see if the different microbes were affecting the nutritional state of the children, the scientists transplanted fecal matter from these twins into the guts of mice raised previously under sterile conditions. Then they fed the mice a diet based on foods eaten in Malawi prior to a two-week treatment with the ready-to-use therapeutic food, followed by resumption of the Malawian diet. The mice transplanted with the malnourished twins' microbes lost much more weight than the mice transplanted with microbes from the well-nourished twins. Similar to the human twins, mice transplanted with the malnourished twins' fecal samples harbored different types of bacteria, some of which have been linked to bowel disease, from those transplanted with material from the well-nourished twins. After the nutritional therapy, the mice with the malnourished twins' microbes showed a shift to more healthy types of bacteria along with indicators of improved nutrition and metabolic function. However, after being put back on the Malawian diet for only four weeks, these indicators fell back to pre-treatment levels. This study's finding—that the combination of dietary deficiency with a particular gut microbial profile causes severe malnutrition—has far-reaching implications for developing sustainable interventions for childhood malnutrition. For example, biomarkers of microbial metabolism may need to be taken into account for designing effective nutritional interventions in some individuals. These and other insights will be helpful in developing more effective approaches to treating and preventing the huge global challenge of severe malnutrition in children.

Smith MI, Yatsunenko T, Manary MJ, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339: 548-554, 2013.

Gut Microbes from People Can Transmit Obese or Lean Body Types to Mice: A study in mice has found that gut microbes obtained from obese or lean people, within certain dietary contexts, can transmit obesity or leanness to mice in the lab. While there is ample evidence that genetics and other factors play an important role in the development of obesity, there is also evidence that the community of bacteria living in the gut and their collective bacterial genomes, or "gut microbiome," may affect and reflect a person's health and nutritional status. For example, recent research raised the possibility that differences in the gut microbiome may explain why twins with identical genetic makeups can have very different disease and nutritional states.

A group of researchers were interested in exploring this idea using sets of identical twins who were "discordant" for obesity, which means one twin was obese while the other was not. Previous research with such discordant twins has shown that obesity is associated with changes in the types of bacteria in the gut; however, it is unclear whether these changes in the gut microbiome actually contribute to the development of obesity. To examine this possibility, scientists transferred the gut microbiomes from twins discordant for obesity into mice previously raised in sterile conditions and initially free of any gut microbes. Even though all mice were fed the same diet, only the mice that received the obese twin's microbiome gained weight, while the mice that received gut microbes from the lean twin did not.

Knowing that mice often share gut microbes with their cage mates, the scientists housed the "lean" mice—those inhabited by the lean human twin's gut microbes—together with the now "obese" mice—those with the obese twin's gut microbes—to see if the microbes from one set of mice would spread to, and affect, the other set of mice. Under these conditions, the weights of the lean mice did not change, but after several days the obese mice lost a significant amount of weight and began to harbor the same types of bacteria that were in the lean mice. This finding suggested that the microbiome from the lean mice, along with its lean-promoting effects, was being transferred to the obese mice, but not *vice versa*.

When the scientists compared the microbiome from the lean mice to that of the obese mice, they found differences in genes that regulate metabolism, including the metabolism of certain amino acids (components of proteins) and effects on fats and starches, suggesting that metabolic changes are responsible for the microbiome's effects on weight. However, the protective effects of the lean twin's microbiome were only seen when the mice were fed a healthy diet with high amounts of fruits and vegetables and low amounts of saturated fat, meaning that changes in weight were not dependent on the microbiome alone, but were also dependent on diet.

These studies provide convincing evidence that the gut microbiome, in conjunction with diet, can strongly affect the ability to gain or lose weight in mice, and may lead to insights into the role of the gut microbiome in regulating weight in humans.

Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341: 1241214, 2013.

Molecule Critical for Gut Pathogen Survival Visualized: Researchers have revealed the structure of a protein complex important for the survival of a species of bacteria that causes gastric ulcers and other gastrointestinal diseases. *Helicobacter pylori* (*H. pylori*) is a species of bacteria that is commonly found living in the human stomach. It is thought to damage the mucus coating that protects the stomach lining, exposing it to powerful digestive acids. This can result in gastric ulcers, which are painful sores that can be accompanied by such symptoms as weight loss, vomiting, and bleeding. Antibiotics are typically used to treat *H. pylori* infections, but they are not always successful and can cause the bacterium to develop antibiotic resistance. This bacterium may have an Achilles' heel, however, as it can only survive the harsh environment of the stomach by neutralizing the potent stomach acids that would otherwise digest the bacterium. It does this by taking up a molecule called urea through a selective opening, a "channel," on the surface of the bacterium. Once inside, the urea is converted into ammonia, which is then used to neutralize the stomach acid. This channel is an ideal target for drugs that can block the entry of urea into the bacterium, thereby threatening its survival, and determining the three-dimensional structure of the channel is necessary before drugs can be designed efficiently.

To obtain the structure, a group of scientists undertook the extremely difficult task of collecting large amounts of the protein complex that creates the channel, purifying it, and condensing it into crystals. They were then able to deduce the structure of the complex by analyzing the pattern made by X-rays after they passed through the crystals, a technique called

X-ray diffraction, the same approach that was used to discover the double helix structure of DNA. The result of the study was the generation of an elegant, three-dimensional model of the channel that *H. pylori* uses to stay alive inside the stomach. The scientists were then able to identify the precise segment that is required for urea entry by changing parts of the channel and testing its ability to take up urea. Now that the detailed structure and critical features of the channel have been defined, scientists can design specific drugs that will block its function, like designing a plug to fit into a hole, thereby preventing the entry of urea into *H. pylori* and, ultimately, impeding its survival in the stomach. This would provide a new strategy to combat this pathogen and open the possibility of new treatments for the prevention of ulcers.

Strugatsky D, McNulty R, Munson K, et al. Structure of the proton-gated urea channel from the gastric pathogen Helicobacter pylori. *Nature 493: 255-258, 2013.*

People and Their Gut Microbes Are Life-long Partners: Scientists have found that the types of bacteria in our gut change very little throughout our lives. The human microbiome, or the collection of microbes present in the body, includes trillions of bacteria living in our gastrointestinal tract. There is evidence that the composition of the gut microbiome can affect human health. One common approach to study the gut microbiome is to sequence the DNA of the bacteria living there, but this method has been limited by the sensitivity and accuracy of the DNA sequencing technique, which had difficulty discerning between individual types or strains of bacteria and errors in the sequencing process. To overcome this limitation, a group of researchers developed a new way to sequence the DNA of gut bacteria that improves the accuracy to a point where the scientists can differentiate between individual bacterial strains. Using this method to sequence DNA contained in stool samples from healthy adults over time, the scientists found that the relative amount of each bacterial strain changes very little over several years. In other words, the demographics of the gut microbial community are likely stable in healthy individuals for decades or longer. The researchers also compared the microbial communities of family members and found them to be much more similar than the microbiota of unrelated people. This finding suggests that family members are colonized by similar bacteria through exposure to a shared environment and that the gut microbiome is established relatively early in a person's life—probably within a few years after birth.

The researchers did find one situation that will disrupt the stability of the microbiome, however: when someone undergoes a drastic change in diet that causes substantial weight loss. A group of volunteers consumed a calorie-restricted liquid diet for several months and subsequently lost about 10 percent of their body weight. The stability of the gut microbiome quickly broke down under these conditions, and the individuals whose weight fluctuated the most also had the least stable microbiomes. Overall, these studies suggest that the types of bacteria in the gut microbiome will remain relatively constant throughout a person's life, though this stability is affected by changes in diet and weight.

This points to the gut microbiome as a potential predictor of an individual's state of health, such that changes in the microbiome could be used in the future as markers for overall well-being and disease development.

Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. Science 341: 1237439, 2013.

IRRITABLE BOWEL SYNDROME

Brief Psychological and Educational Therapy Improves Symptoms of Irritable Bowel Syndrome: Researchers have found that patients with irritable bowel syndrome (IBS) show an improvement in symptoms following a short course of group therapy involving psychological and educational approaches. IBS is a collection of symptoms, including abdominal pain or discomfort (such as cramping), along with diarrhea, constipation, or both. A primary reason why IBS is difficult to treat is that its exact causes are not well understood, although it is believed to have both

physical and mental origins. One possible cause is a problem with communication between the brain and the gut, which could lead to changes in bowel habits. Based on this likely mind-body connection, some successful treatment of IBS has been achieved using psychological counseling and education-based therapies to help patients control the activity of their own nervous systems and gastrointestinal tracts, but variable results, along with cost issues, unavailability of trained clinicians, and a general preference for pharmaceutical remedies, have hindered the implementation of psychological therapy as a standard of treatment.

To determine if a combination of psychological and educational therapy can lead to a sustained improvement in IBS symptoms and an increase in the quality of life for patients, a team of scientists performed a clinical trial where IBS patients in the intervention group attended a five-week series of two-hour group classes co-led by a gastroenterologist and therapist to promote self-efficacy and teach relaxation techniques. As a basis for comparison, a control group of patients was monitored while on the waiting list for the group classes. Patients who attended the group classes learned about the linkage between emotions, stress, and abdominal symptoms with an emphasis on their ability to control the activity of their own bodies. They were also taught about the connection between mood, stress, and GI symptoms, and the difference between ineffective coping styles, such as panicking during moments of anxiety, and more effective responses, such as arriving at conscious, rational solutions during stressful situations. The classes also instructed patients on deep breathing techniques and progressive muscle relaxation, including homework assignments consisting of at least 15 minutes of relaxation exercises twice a day. During and after the trial, the class participants and control patients were asked to monitor and document their symptoms in relationship with their mood states, stressors, and diets. The results of this trial suggested that patients who underwent the group psychological and educational therapy had a reduction in IBS symptoms and a better quality of life, lasting for at least three months after the trial, than those who did not participate in the class. The therapy was particularly helpful for those individuals who had a low or average quality of life prior to starting the intervention, although it had less of an effect for those whose quality of life was higher at the beginning of the study. This study demonstrated an effective, low-cost method of treating IBS symptoms, especially for those with low or average health-related quality of life, and could pave the way for the adoption of such an approach as an alternative to, or a supplement for, pharmacological therapy.

Labus J, Gupta A, Gill HK, et al. Randomised clinical trial: symptoms of the irritable bowel syndrome are improved by a psycho-education group intervention. Aliment Pharmacol Ther 37: 304-315, 2013.

INFLAMMATORY BOWEL DISEASE

New Insights into the Mechanism of Intestinal Lining Repair: Researchers have discovered a new role for a protein called Wnt5a in the regeneration of damaged gut tissue. The human intestinal tract is prone to injury by conditions such as inflammatory bowel disease, where the lining of the gut is damaged as a result of chronic inflammation and irritation. An important component of the intestinal lining is a collection of specialized structures called "crypts," which are small depressions in the lining of the gut. At the base of these crypts are intestinal stem cells, which replenish the injured lining by proliferating and maturing into specific cell types needed to replace the damaged tissue.

Intestinal crypts are damaged during injury to the lining, but how these vital structures are replaced is unclear. A group of scientists set out to address this question using a mouse model of simulated intestinal injury by surgically removing some of the crypts in the mouse's gut, then examining the intestinal tissue both within and nearby the damaged site. They found that cells from intact crypts near the damaged site will migrate into the wound and form a series of shallow channels, which eventually subdivide into new crypts to replace the damaged ones. When the scientists

examined some of the cells in these channels, they found a high abundance of a protein called Wnt5a, which suggested that it might be important for crypt regeneration. To test this idea, the researchers looked at intestinal tissue healing in a mouse model that is missing Wnt5a from its intestinal cells. When the intestinal lining of these mice was injured, no new crypts were formed, confirming that Wnt5a was necessary for the renewal of these structures. The scientists were then interested in exactly how Wnt5a was guiding the formation of new crypts, so they looked at changes in intestinal cells in culture upon exposure to this protein. They found that the cells stopped proliferating and formed small channels, similar to what happened in the mouse model. Further analysis of the cells exposed to Wnt5a showed that this protein activates a pathway that is known to stop cell proliferation, providing insight into the mechanism by which Wnt5a supports crypt regeneration. This research sheds light on how intestinal tissue is repaired after injury, and could lead to new ways to help facilitate the regeneration of intestinal cells that are damaged in diseases like inflammatory bowel disease.

Miyoshi H, Ajima R, Luo CT, Yamaguchi TP, and Stappenbeck TS. Wnt5a potentiates TGF-β signaling to promote colonic crypt regeneration after tissue injury. Science 338: 108-113, 2012.

Intestinal Inflammation Works Through Microbes To Raise Cancer Risk: Researchers have uncovered a surprising interaction between intestinal inflammation and a type of bacteria that promotes colorectal cancer (CRC). Chronic intestinal inflammation is considered a risk factor for CRC, although the process by which it increases risk is unclear. The human intestine is also home to trillions of microbes, which reside near the intestinal cells affected by inflammation and cancer. One research group questioned whether these microbes were innocent bystanders, or if they played a role in the transition from inflammation to cancer within the intestine. First, they sequenced genetic material from bacteria in intestinal and stool samples taken from a mouse model of intestinal inflammation that was genetically programmed to develop colitis (colonic inflammation). They also analyzed samples from control, non-altered mice. The sequencing showed that while the total numbers of intestinal bacteria were similar, the types of microbes found inside the colon differed in mice predisposed to colitis compared to controls. One type of bacteria in particular that was more abundant in the mice with colitis was *Escherichia coli* (*E. coli*), which is also more plentiful in the colons of humans with inflammatory bowel disease (IBD) and CRC. When the mice were given a chemical carcinogen, only those predisposed to colitis developed cancer. Next, the researchers inoculated mice raised under germ-free conditions with a single type of intestinal bacteria—either *E. coli* or another type of bacteria called *Enterococcus faecalis* (*E. faecalis*). The colitis-prone mice inoculated with *E. coli* and given the chemical carcinogen developed an invasive form of colon tumors, while the mice inoculated with *E. faecalis* and given the carcinogen were less likely to develop invasive tumors. Searching through the *E. coli* bacterium's genome, the researchers identified a gene in some bacterial strains coding for a protein that damages DNA. They found that a strain of *E. coli* with this toxic gene was more likely to damage DNA in an intestinal cell line compared to another *E. coli* strain lacking this gene. Likewise, infection of the colitis-prone mice with this toxic strain of *E. coli* resulted in more numerous and invasive tumors than those in mice infected with the less-toxic strain. The researchers also found this strain of *E. coli* with the DNA-toxic gene in samples taken from patients with IBD and CRC. This study provides evidence that certain types of intestinal microbes are key players in the complex transformation of colon cells from an inflamed to a cancerous state. Future research will likely focus on other bacterial strains that may contribute to this progression from intestinal inflammation to CRC.

Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338: 120-123, 2012.

Particles Released from a Gut Microbe Protect Against Colitis: A team of scientists has discovered how a species of gut bacteria interacts with the immune system to suppress inflammatory bowel disease (IBD),

in a study in mice. IBD is the general name for the diseases, such as Crohn's disease and ulcerative colitis, that cause symptoms such as abdominal pain and diarrhea due to inflammation and irritation in the intestines. *Bacteroides fragilis* (*B. fragilis*), a species of bacteria that inhabits the human intestinal tract, can suppress inflammation in diseases like IBD. This type of bacteria makes a molecule called polysaccharide A, which not only protects the bacteria from the harsh environment of the intestine, but also activates a group of immune cells called regulatory T cells that inhibit inflammation by restraining the immune response. However, exactly how *B. fragilis* interacts with the immune system through this bacterial molecule was a mystery.

A group of researchers tackling this question found that polysaccharide A is released from cultures of *B. fragilis* in small spheres, called outer membrane vesicles (OMVs), which bud from the bacterial cells' outer coating. When given to mice orally, these OMVs prevented an experimental form of colitis. When OMVs were added to immune cells in laboratory culture, polysaccharide A was found to accumulate in a type of immune cell called a dendritic cell, which communicates with regulatory T cells to activate them. This is particularly important in the gut, because dendritic cells have the key role of sampling intestinal contents to coordinate T cell reactions. When OMVs were added to cultures containing both dendritic cells and T cells, there was an increase in the production of an anti-inflammatory chemical produced by the T cells. The researchers predicted that a dendritic cell protein called TLR2, which senses bacterial products, was necessary for this response. Indeed, when OMVs were added to dendritic cells that were lacking TLR2, the dendritic cells did not cause an increase in the anti-inflammatory chemical in T cells. In support of this, the scientists found several TLR2-dependent genes that were turned on in dendritic cells by polysaccharide A, including a gene called *Gadd45a*, which is required to promote T-cell response. Mice that were genetically manipulated to lack the *Gadd45a* gene were not protected against colitis when they were treated with OMVs, unlike the non-genetically manipulated mice. This study demonstrates a fascinating interaction on a molecular level between a gut microbe and the immune system of its mouse "host" that leads to protection against disease. More importantly, it opens the possibility for future research in humans that could potentially lead to new therapies for inflammatory bowel disease.

Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, and Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe 12: 509-520, 2012.

LIVER METABOLIC FUNCTION AND DISEASE

Tiny RNA Has Big Effects on Lipid Metabolism and Atherosclerosis: Scientists have shown that a small molecule, called "microRNA," plays a big role in lipid (fat) metabolism and related health conditions such as hyperlipidemia (elevated lipid levels in the blood) and atherosclerosis (clogging and hardening of the arteries). High levels of lipids circulating in the blood put individuals at higher risk for cardiovascular and metabolic disorders. Lipids are carried in the blood in packages called lipoproteins, which are assembled, mostly in the liver, with help from proteins such as the microsomal triglyceride transfer protein, or MTP. Researchers aimed to determine whether there were any regulators of MTP in the body that might be targets for lowering elevated blood lipids. MicroRNAs or "miRNAs" are small pieces of RNA that target another type of RNA, abbreviated mRNA (for messenger RNA), which is involved in protein production—ultimately, reducing the amount of protein made from the mRNA.

The research team looked at several miRNAs that could potentially regulate MTP and identified one—miR-30c—that reduced MTP activity, as well as decreased the concentration of lipoproteins secreted in a cell culture model. They found that miR-30c accomplishes this feat by binding to and degrading MTP mRNA. To test the miRNA's effects on physiological functions, they moved to an animal model—mice that were injected with a substance to boost miR-30c levels in the liver, in particular. After three weeks, the mice had reduced levels of MTP in the liver, as well

as cholesterol in the blood, reflecting that amounts of certain lipoproteins were also lowered. Surprisingly, the abundance of miR-30c not only inhibited the packaging of lipids into lipoproteins, but it also inhibited the synthesis of the lipids themselves, suggesting an even greater role for this miRNA in lipid metabolism. Mice with extra miR-30c in the liver also had fewer, smaller plaques in their arteries; large amounts of arterial plaques are a sign of atherosclerosis. The results of this study outline the key role of this specific miRNA in reducing lipid synthesis, secretion of specific lipoproteins, and, ultimately, atherosclerosis. These findings highlight the potential importance of agents that could boost or mimic the effects of this miRNA as a treatment for those at risk for cardiovascular and metabolic disorders.

Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, and Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nat Med 19: 892-900, 2013.

Cell Signaling Pathways Point to Top Notch Solution for Fatty Liver Disease: Scientists have identified a cell signaling pathway that may contribute to the development of fatty liver disease and may also point to therapeutic solutions. Obesity is associated with several metabolic diseases, including type 2 diabetes and nonalcoholic fatty liver disease, or "NAFLD," which is marked by fat accumulation in the liver. Treatment options for NAFLD are particularly limited. One mystery surrounding these obesity-associated metabolic diseases has been how some insulin-responsive pathways in liver cells, such as fat production, continue to function when insulin is elevated—ultimately, contributing to fatty liver—while others, such as suppression of glucose production, become resistant to the action of insulin. Researchers explored cell signaling pathways that could be responsible for the overproduction of fat in the liver associated with insulin resistance. They narrowed their search to a signaling pathway initiated at a receptor protein spanning the cell surface known as "Notch," which regulates some metabolic functions in the liver, but is also well-known for its role in early development. After feeding mice a high-fat diet, the team saw greater Notch activity in the liver, along with more fat deposition in the organ. However, when mice were genetically altered to lack a key factor in the liver needed for Notch signaling, their livers showed less fat accumulation than in the non-genetically altered mice. Similar results were observed when Notch signaling was again disabled, this time in mice with a defective Notch receptor protein. To gather more evidence for their theory, they also created a mouse model in which Notch signaling was active all the time. In this model, they observed fat accumulation in the liver even though the mice were given a more balanced (non-high-fat) diet. Using the expression of some genes that are typically affected by the Notch signaling pathway as a readout, the scientists determined that Notch signaling acts through a molecule within the cell called mTorc1 to increase fat production in the liver. If studies in humans show a similar role for Notch signaling, this could be an important therapeutic target to block in treating NAFLD associated with obesity. This finding is especially promising in light of the fact that agents to inhibit Notch signaling are already available and being tested in clinical trials against other diseases.

Pajvani UB, Quang L, Kangsamaksin T, Kitajewski J, Ginsberg HN, and Accili D. Inhibition of Notch uncouples Akt activation from hepatic lipid accumulation by decreasing mTorc1 stability. Nat Med 19: 1054-1061, 2013.

Treatment To Prevent Hepatitis C Recurrence After Liver Transplantation: A clinical trial conducted at U.S. liver transplant centers has shown that pre-treatment of patients infected with hepatitis C, using antiviral therapy to suppress the viral infection, can prevent recurrence of the infection once patients are transplanted with a healthy donor liver. Chronic hepatitis C is one of the major reasons for adult liver transplantation. Adult-to-adult living donor liver transplantation—removal of part of a living adult's healthy liver for transplantation into another adult with liver disease—is one option for patients whose livers have been damaged by hepatitis C. However, in patients who still have hepatitis C virus (HCV) circulating in their blood at the time of transplantation, the infection will inevitably recur and threaten the health of the transplanted organ, whether from a living or cadaveric donor. Previous small studies had suggested one strategy to prevent this is with treatment prior to transplant using antiviral drugs against

HCV—pegylated interferon and ribavirin—to suppress viral levels in the blood. But this antiviral treatment is less effective in individuals with some types of the virus and can cause side effects.

Researchers at seven U.S. transplant centers participating in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) performed the first randomized clinical trial assessing the efficacy and safety of antiviral therapy pretreatment to prevent hepatitis C recurrence after liver transplantation. Patients infected with some of the HCV types prevalent in the United States were recruited into treatment and control groups. Prior to transplant, the treatment groups were started on a low dose of peginterferon and ribavirin that steadily increased over the course of treatment, which lasted up to a maximum of 48 weeks. The researchers measured viral RNA levels to determine whether the virus was responding to treatment; an undetectable level of viral RNA in the blood after several weeks of treatment, before then after transplant, represented a response. They found that patients who were treated prior to transplant for at least 16 weeks were more likely to have undetectable levels of HCV after transplant than those with shorter treatments. Overall, a similar number of total adverse events occurred in the treated and control groups, although the number of adverse events per person and also incidence of the particular adverse event of bacterial infection were higher in the treated groups. This study provides clinical evidence that antiviral therapy prior to a liver transplant can prevent hepatitis C recurrence in some patients. This finding is particularly applicable to patients who are better able to tolerate an extended course of antiviral therapy and its potential side effects.

Everson GT, Terrault NA, Lok AS, et al. A randomized controlled trial of pretransplant antiviral therapy to prevent recurrence of hepatitis C after liver transplantation. Hepatology 57: 1752-1762, 2013.

Biliary Atresia Susceptibility Gene Identified in Patients: Scientists participating in NIDDK's Childhood Liver Disease Research and Education Network (ChiLDREN) have utilized patient samples and an animal model to identify a genetic deletion that may play a role in the development of biliary atresia. Biliary atresia (BA) is a life-threatening condition affecting newborn infants in which the bile ducts—tubes that carry bile from the liver to the gallbladder for storage and to the small intestine to aid fat digestion—become blocked and destroyed, leading to a back-up of bile and liver damage. Most infants with biliary atresia require surgery to survive and may later need a liver transplant as well. Although its causes are unknown, some have speculated that it results from a combination of environmental and genetic factors. A group of researchers at a site participating in the NIDDK's ChiLDREN Network aimed to build on past findings of an association between a region of the genome and BA by looking for a specific gene(s) within this region that confers increased disease risk. By comparing genetic sequences from patient blood samples at the Network site with healthy controls, they zeroed in on a small part of this genetic region that was deleted in the patients. In healthy individuals, this region contains only one gene, called *GPC1*, coding for a protein involved in organ development. To more directly observe the functional impact of this gene's deletion on biliary development, they turned to an animal model with an analogous form of the gene (*gpc1*) and similar biliary anatomy—the zebrafish. In this model, the research group could use molecular inhibitors to reduce activity of the fish's *gpc1* gene early in development, mimicking the deletion of this gene in the patients with BA. They observed defects in biliary anatomy in the fish larvae with reduced *gpc1*, but not in the control larvae. Applying this knowledge to the human disease, they looked for the GPC1 protein in liver samples from patients with BA and healthy controls. The livers of patients with BA showed lower levels of GPC1 compared to the livers from healthy controls. This study identifies *GPC1* as a new potential susceptibility gene that may be lacking in patients with BA and shows the mechanism in an animal model by which this gene deletion may result in human disease. The ChiLDREN Network has enabled studies of BA such as this one through collecting samples from a sufficient number of patients affected by this relatively rare disease.

Cui S, Leyva-Vega M, Tsai EA, et al. Evidence from human and zebrafish that GPC1 *is a biliary atresia susceptibility gene. Gastroenterology 144: 1107-1115, 2013.*

Drug-Induced Liver Injury Network

Drug-induced liver injury, though relatively uncommon in the United States, represents the leading cause of acute liver failure in the nation and the top reason for regulatory actions by the U.S. Food and Drug Administration against approved medications. Products on the market implicated in causing liver injury currently include several hundred prescription drugs, over-the-counter medications, herbal products, and dietary supplements. However, knowledge has been limited concerning the scope of this problem in the U.S. population, mechanisms by which susceptible individuals develop drug-induced liver injury, or how to definitively diagnose these cases. Testing of new drugs in pre-clinical and clinical trials before they are approved and reach the wider patient population does not always provide reliable information on their potential risk of causing liver injury. Diagnosis of drug-induced liver injury can also be complicated by similarities to other forms of liver disease and variability in responses across patients—currently, it is a "diagnosis of exclusion," made only after all other possible causes have been ruled out.

The NIDDK established the Drug-Induced Liver Injury Network (DILIN) in 2003 to collect and analyze cases of severe liver injury caused by prescription drugs, over-the-counter drugs, and alternative medicines, such as herbal products and supplements. Since that time, DILIN has collected more than 1,000 cases of liver toxicities due to these agents and made major contributions to characterizing potential mechanisms and disease processes; defining the clinical spectrum, natural history, and outcomes of liver injury that results from these agents; and aiding accurate diagnosis. For example, studies of data generated by DILIN have informed the use of assessment tools used for diagnosing drug-induced liver injury and have also characterized the pathological and clinical features, as well as outcomes resulting from this form of injury. Additionally, the Network has found that, over time, the number of enrolled cases of liver injury attributed to herbal and dietary supplements, particularly supplements used for bodybuilding, has steadily increased, now representing the second most commonly recorded class of liver toxicity-causing products in the Network. Analyses of Network data also yielded the surprising finding that some cases previously diagnosed by physicians as drug-induced liver injury in the United States were attributable instead to infection with the hepatitis E virus.

An additional benefit of DILIN came in 2012 in the form of a website called "LiverTox," which was developed by the NIDDK in conjunction with the NIH's National Library of Medicine. This website features sample cases of drug-induced liver injury based on the Network data, as well as a database with summaries of liver injury reports for a given drug or herbal/dietary supplement. The website serves as a public resource to aid health care providers in diagnosing, and investigators in studying, liver injury due to drugs and herbs/supplements. Additional information on LiverTox is available at: *http://livertox.nih.gov/*

In 2013, the NIDDK announced a new initiative to continue and expand the Network and the studies it enables. The new initiative will have several objectives for the renewed Network, including: to enhance enrollment of individuals who experienced drug-induced liver injury from diverse backgrounds and geographic distributions; attempt to enroll patients as early as possible after injury to better characterize disease processes; develop an accurate and user-friendly computer-based instrument for improved diagnosis; continue clinical and genetic analyses to determine the role of genetic variability in this form of liver injury; support studies of disease processes and potential means of prevention or treatment; and continue support for the LiverTox website as a resource for collecting additional cases from the medical community. The renewed Network will be composed of up to five clinical centers across the country with expertise in diagnosis and management of drug-induced liver injury and a data coordinating center. Once the new Network is formed, an invitation will be sent to all academic medical centers in the United States to identify and document cases of drug-induced liver injury at their sites, using the online case report system developed by the Network investigators, the NIDDK, and the NIH's National Library of Medicine. In this way, this unique Network will be strengthened in its efforts to yield new insights into diagnosis, treatment, and, ultimately, prevention of drug-induced liver injury.

Fecal Incontinence Workshop

On August 19-20, 2013, the NIDDK hosted a workshop, "Developing a Clinical Research Agenda for Fecal Incontinence," to address the gaps in clinical and basic research for this condition that affects nearly 18 million adults[1] and represents a significant personal and public health burden, with considerable economic cost. The speakers included a panel of experts in epidemiology, gastrointestinal physiology, gastroenterology, colorectal surgery, urogynecology, psychology, and behavioral medicine. During the workshop, the panel identified and discussed major issues in the diagnosis and treatment of fecal incontinence, and they also discussed future directions to advance progress toward developing treatment strategies. Current therapies for the treatment of fecal incontinence were reviewed, including biofeedback, surgical repairs, and a gel, recently approved by the FDA, that is injected under the tissues surrounding the anus to tighten the anal opening.

A major theme of this meeting was the formidable challenge of raising the public awareness of this underdiagnosed condition. The panel raised concerns surrounding the lack of screening by health care providers, and the emotional and sociological difficulties that patients face in deciding to come forward and talk about this condition so they can get treatment. These hurdles were illustrated during a courageous testimony from a patient who shared her experiences living with fecal incontinence. Recommendations from this workshop will help guide future research on this debilitating condition.

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[1] *Whitehead WE, Borrud L, Goode PS, et al. Fecal incontinence in U.S. adults: epidemiology and risk factors. Gastroenterology. 137: 512–517, 2009.*

STORY OF DISCOVERY

Genetic Insights into Pancreatitis

Since the discovery in the 1990s of the first genetic risk factor associated with pancreatitis by an NIDDK grantee, subsequent studies supported by the Institute have identified a number of genetic variants associated with this disease. Pancreatitis is a disease marked by inflammation of the pancreas. A small gland located near the small intestine, the pancreas is responsible for producing enzymes that, mixed with bile from the gallbladder, aid in the digestion of food. In a healthy pancreas, these enzymes are released in an inactive form, to become activated only when they reach the intestine. However, when the pancreas is inflamed, as in pancreatitis, these enzymes become activated while still within the pancreas, where they degrade the very tissue that produced them, causing episodes of pain ranging from mild to severe, as well as nausea and vomiting. Pancreatitis can be acute, with inflammation resolving within a few days, or chronic, involving long-term inflammation and tissue damage. Over time, chronic pancreatitis leads to permanent damage to the pancreas and an increased risk of pancreatic cancer, one of the most devastating of all malignancies. Currently, there are no cures or preventive therapies for pancreatitis. But, investigations into how pancreatitis develops, including genetic variants that can contribute to this disease, have the potential to improve diagnosis, prevention, and treatment.

A variety of factors may contribute to the development of pancreatitis, often interacting in complex ways, including genetics, gallstones, heavy alcohol use, and other causes; unique combinations of factors may occur in different people. NIDDK-sponsored research has led to advances in the discovery of genetic factors associated with hereditary, chronic, acute, and other forms of pancreatitis. For example, the North American Pancreatic Study 2 (NAPS2) is a multi-center clinical study building on past research to uncover additional genetic markers that may help to identify individuals susceptible to pancreatitis and prevent the disease from developing. Genetic testing for mutations in pancreatitis-related genes based on this knowledge has the potential to provide information not only on disease risk, but also causes, severity, and likelihood of progression.

Following are a few highlights of NIDDK-sponsored research advances over the past few decades that have contributed to an explosion of new knowledge regarding the role of genetic factors in pancreatitis.

Trypsin-related Genetic Factors

In 1996, scientists identified the first pancreatitis-related gene mutation in patients with a rare genetic form of the disease called hereditary pancreatitis. This gene coded for the protein cationic trypsinogen, an inactive precursor form of the digestive enzyme trypsin. Trypsinogen is produced by cells in the pancreas then secreted into the small intestine, where it becomes activated to trypsin, an enzyme that breaks down protein and activates other digestive enzymes. Normally, the body has a fail-safe mechanism whereby trypsin is programmed to self-destruct if prematurely activated while still inside the pancreas. However, the mutations in the trypsinogen gene found in patients with hereditary pancreatitis disable this mechanism, enhancing trypsin activation in the pancreas, where it causes cell damage and pancreatitis.

Since that initial groundbreaking discovery, additional mutations associated with pancreatitis were identified in the trypsinogen gene, as well as in other genes that

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relate to trypsinogen/trypsin function. Mutations in the gene associated with cystic fibrosis—the *cystic fibrosis transmembrane conductance regulator* gene (*CFTR*)—were linked in 1998 to the pancreatitis of unknown cause that develops *in utero* in these patients. *CFTR* codes for an ion channel that plays an important role in maintaining the fluid that flushes enzymatic precursors like trypsinogen out of the pancreas and into the small intestine. In 2000, mutations in the *pancreatic secretory trypsin inhibitory* gene (*PST1/SPINK1*), which encodes a trypsin inhibitory protein, were identified in patients with pancreatitis that cause a loss of this inhibitory function, disabling this first line of defense against prematurely activated trypsin in the pancreas. Later, additional genes coding for proteins that affect trypsin activation, such as the *chymotrypsinogen C* gene and *calcium sensing receptor* gene, were also found to contribute to pancreatitis susceptibility.

New Genetic Variants

Recent findings of genetic studies on pancreatitis have pointed to other, non-trypsin-related mechanisms that offer insights into disease development and potential targets for prevention or therapy.

In 2012, the first genome-wide association study of pancreatitis identified a new gene associated with pancreatitis. Using samples collected by the NAPS2 Study from individuals with recurrent acute pancreatitis, chronic pancreatitis, or healthy controls, the researchers scanned their genomes to identify associations with variations in two genetic regions—one in the *trypsinogen* gene and another in a gene called *claudin-2*. Claudin-2 is a tight junction protein that regulates the movement of ions and water. The *claudin-2* gene variant was strongly associated with chronic pancreatitis, particularly in disease resulting from alcohol abuse in men. This finding could explain, in part, why most patients with alcohol-related pancreatitis are men.

Recently, researchers again used samples from the NAPS2 Study to identify mutations in a *gamma-glutamyltransferase 1* (*GGT1*) gene associated with chronic pancreatitis; these same mutations had been previously linked to pancreatic cancer. The protein formed from this gene typically maintains glutathione levels, which protect cells against damage by free radicals and oxidation. This discovery suggests another mechanism through which pancreatitis may occur—one which may lead not only to inflammation, but also to cancer formation in the pancreas.

Future Directions in Pancreatitis Research

The Institute continues to identify areas of new research opportunity related to pancreatitis, such as genetic risk factors, with help from stakeholders in the external research, professional, and patient advocacy communities.

In June 2012, the Institute convened a two-day research workshop at the NIH campus in Bethesda, Maryland, entitled "Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair." This workshop provided an update on a wide range of research efforts addressing acute and chronic pancreatitis, including susceptibility genes, and charted a course for advancing future research in this area by addressing current gaps and opportunities. Organizers included NIDDK staff, members of the NIDDK National Advisory Council who are active in this area, and the National Pancreas Foundation, as well as others in the external pancreatitis research community. Presenters from around the globe shared their findings, representing research institutions from the United States, England, Canada, Germany, and Spain. Participants also shared research resources, such as the "Pancreapedia" website, which is supported in part by the NIDDK (*www.pancreapedia.org*).

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The proceedings of this workshop continue to guide investigators toward promising future research directions in this area. Consistent with one of its objectives, a report summarizing the meeting was published in the January 2013 issue of the journal *Gastroenterology*, in order to share knowledge and recommendations with the larger research community focused on advancing understanding and improving care for those with acute and chronic pancreatitis. NIDDK staff also published an article summarizing gaps and opportunities in pancreatic disease research identified in this workshop in the May 2013 issue of the journal *Pancreas*, to reach a broader portion of the research community and encourage their applications for available funding mechanisms to boost research in this area.

In June 2013, the NIDDK and National Cancer Institute co-sponsored a two-day workshop at the NIH campus on "Pancreatitis, Diabetes, Pancreatic Cancer." The purpose of the workshop was to examine risk factors contributing to the development of pancreatic cancer, to address clinically relevant questions regarding treatment and surveillance of at-risk populations, and to identify opportunities and priorities for further research.

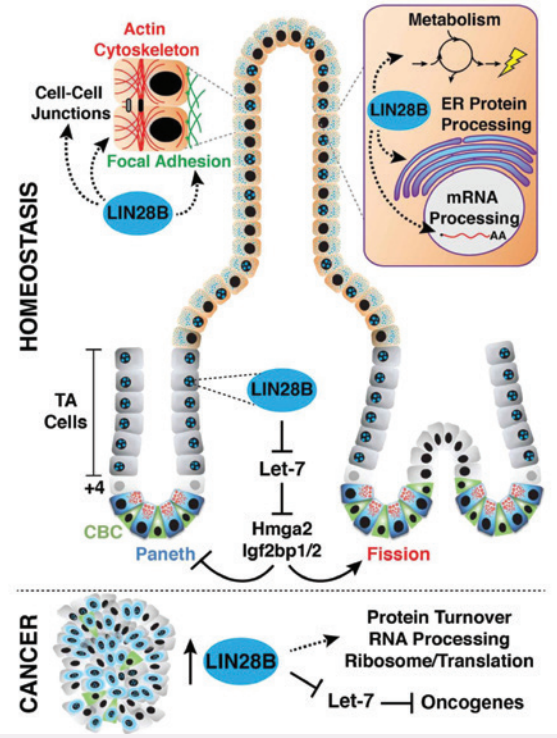
Through ongoing NIDDK studies of pancreatitis, such as NAPS2 and the Study of Nutrition in Acute Pancreatitis, as well as new efforts with its research partners, the story of genetic factors in pancreatitis is still being written. And, with this research, chances are steadily increasing for a happy ending where those at risk from pancreatitis are identified and the disease is effectively managed.

SCIENTIFIC PRESENTATION

The Yin and Yang of Intestinal Stem Cell Biology

Dr. Anil Rustgi

Dr. Anil Rustgi is the T. Grier Miller Professor of Medicine and Genetics and Chief of the Division of Gastroenterology at the University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania. Dr. Rustgi earned his B.S. from Yale University and his M.D. from Duke University. Following a medical internship and residency at Beth Israel Hospital, Harvard Medical School, he completed his fellowship training in gastroenterology at Massachusetts General Hospital, Harvard Medical School, where he rose to the rank of Associate Professor of Medicine. He was Editor-in-Chief of the journal Gastroenterology *(2006-2011), Editor of the textbook* GI Cancers*, and Associate Editor of the textbook* Goldman's Cecil Medicine*. His research focuses on the molecular genetics of gastrointestinal epithelial cellular processes, including those originating from the esophagus, pancreas, and colon, as well as the roles of oncogenes, tumor suppressor genes, tumor initiation and progression, and tumor microenvironment. Dr. Rustgi's work is supported by both the NIDDK and the National Cancer Institute. His academic record includes over 200 publications and 100 book chapters, reviews, and editorials. He is also active in teaching and mentoring the next generation of biomedical researchers at the undergraduate, graduate, and medical school levels, with nearly 25 students and 30 fellows trained in his lab, 10 of whom now have their own independent and leadership positions. Dr. Rustgi presented his laboratory's recent research findings at the February 2013 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.*



This diagram illustrates the complex interactions between molecules such as LIN28B and Let-7 in governing the activities of intestinal stem cells, which are located at the base of structures in the gut lining called "villi." These molecules can influence whether intestinal stem cells engage in everyday activities that maintain the healthy intestine (termed "homeostasis") or escape normal controls, leading to diseases such as cancer. ***Image courtesy of Dr. Anil Rustgi, adapted from an image from Madison, et al. LIN28B promotes growth and tumorigenesis of the intestinal epithelium via Let-7.*** ***Genes Dev*** ***October 15, 2013, 27: 2233-2245, http://genesdev.cshlp.org/content/27/20/2233.full, copyright 2013, Endocrine Society.***

SCIENTIFIC PRESENTATION

Every 5 to 6 days, an amazing feat of self-renewal takes place in the human gastrointestinal tract. Its inner lining, called the intestinal epithelium, completely rejuvenates itself—sloughing off old, damaged cells and replacing them with new ones that carry on the many important intestinal functions such as nutrient absorption and defense against pathogens. To create this new "inner you" repeatedly throughout the human lifespan, intestinal stem cells continually proliferate before "differentiating," or committing to becoming a particular type of intestinal epithelial cell. But, this same process of active proliferation and cell expansion can also have a dark side—intestinal epithelial renewal can go awry in disease states such as cancer, inflammatory bowel diseases, and infectious diarrhea, amongst others.

How do stem cells of the intestinal epithelium balance the light and dark aspects of their unique nature, proliferating just enough to replace worn-out cells without becoming "transformed" into tumors or contributing to other forms of gastrointestinal disease, and what happens at the cellular and molecular levels when this equilibrium is lost? Dr. Rustgi shared findings from his lab's explorations into some of the yin and yang elements of intestinal stem cell biology.

A Tale of Two Stem Cell Populations

The research summarized by Dr. Rustgi was supported in part through the Intestinal Stem Cell Consortium, a team of scientists from research centers across the country established in 2009 by the NIDDK and the National Institute of Allergy and Infectious Diseases to work together toward advancing understanding of intestinal epithelial stem cell biology during development, homeostasis, regeneration, and disease, as well as to aid the development of new therapies for intestinal diseases. Additional support for the research was provided through an NIDDK R01 grant to Dr. Rustgi and his team.

Dr. Rustgi began his story of the stem cells that replenish the intestinal epithelium with a *Fantastic Voyage*-style journey to the inner surface of the intestine, which is covered with fingerlike projections called villi. There at the base of the villi, within an unlikely structure for the birth of new cells called the "crypt," reside what scientists believed for many years was a single population of stem cells that gave rise to all the intestinal epithelial cell types with their unique functions, such as absorptive cells called enterocytes and several kinds of secretory cells. But, using a process that labels stem cells based on their gene products, recent studies by other groups have identified two distinct intestinal stem cell populations. These stem cell populations display different but complementary characteristics: one type proliferates under normal conditions, while the other typically lies dormant until injury or another form of stress triggers its proliferation. Intestinal stem cells are influenced by their local community of neighboring cells and the stroma, or stem cell "niche," as well as the molecular signals passing between them, which are altered by stressors such as infection, inflammation, and cancer. Scientists have also used this genetic technology to track the "daughter cells," or progeny of these stem cells, to better understand intestinal stem cells and their dual nature—their capacity to renew the intestinal epithelium, but also their potential to lead to disease. As part of these investigations, scientists in Dr. Rustgi's lab are focusing on two interdependent molecules that influence the stem cells' fate.

The Dark Side of Stem Cells

One molecule implicated in the intestinal stem cell's journey down the dark path of disease is one that under certain conditions can contribute to cancer; it is called *LIN28*. First discovered in *Caenorhabditis elegans*, a worm commonly used in molecular research, *LIN28* is also present in humans and comes in two forms—a and b. Normally, the LIN28 proteins

bind to some RNA molecules that encode proteins, and inhibit processing of certain other types of RNA molecules, called "microRNAs." MicroRNAs do not code for any proteins themselves, but exert their effects on the production of other RNAs, either by facilitating or inhibiting processing of some RNAs that do code for proteins. Through its interactions with RNAs, LIN28 affects glucose metabolism. It is also famous among scientists as one of several factors that, when added experimentally to adult somatic (nonreproductive) cells, can induce the formation of stem cells.

Dr. Rustgi described work led by Dr. Blair Madison, a Research Associate in the Rustgi lab and the University of Pennsylvania GI Division, who also has an NIDDK K01 grant. Dr. Madison and other scientists in Dr. Rustgi's lab used mouse models to explore the physiological impact of factors such as LIN28. For example, in a mouse model genetically engineered to activate the mouse form of Lin28b at varying levels only in cells of the intestinal epithelium, they observed expanding cell proliferation with high Lin28b levels. The mice also had overgrown or "hypertrophic" intestines and colons, developing colon polyps and then colon cancer as they aged. These results provided evidence supporting Lin28b's cancer-causing properties when present at high levels in the intestine.

However, Lin28b also showed an unexpected relationship to stem cell fate decision-making. Using a stain to visualize cells at the base of the crypts, mice with elevated Lin28b showed a loss of the secretory Paneth cells that interdigitate with the stem cells and release antimicrobial chemicals; however, no simultaneous loss of stem cells was observed. This finding challenged a prevailing theory that Paneth cells were necessary for supporting neighboring stem cells.

Balancing Act

What keeps pro-growth molecules such as LIN28 in check just enough to achieve continuous but controlled intestinal epithelial cell turnover? Balancing the effects of molecules such as LIN28 are forces such as so-called "tumor suppressors" that act as a molecular safety mechanism to balance pro-growth signals in the body. One such tumor suppressor is *let-7*. Also discovered in the *C. elegans* worm, *let-7* is a type of microRNA with the power to control certain genes involved in cell proliferation. *Let-7* is also targeted by LIN28, which inhibits *let-7* formation. LIN28 and *let-7*, therefore, represent opposing forces—LIN28 encourages cell proliferation, while *let-7* inhibits it. Under normal conditions, *let-7* helps keep intestinal stem cells from crossing over to the "dark side" of cancer by suppressing genes that control cell proliferation. However, when there is an overabundance of LIN28, it inhibits *let-7*, effectively taking the brakes off of the cell so that it races ahead with its proliferation program.

For example, when Dr. Madison on Dr. Rustgi's team generated and studied genetically modified mice that were missing *let-7* specifically in cells of the intestine and colon, they observed a rise in pro-growth genes, agreeing with *let-7*'s important role in slowing growth. The team next looked at mice that were genetically altered so that not only were they missing *let-7*, but they were also producing high levels of LIN28b. These mice showed a loss of the secretory Paneth cells along with some intestinal overgrowth or "hypertrophy"—signs that many, but not all, of LIN28b's effects are dependent on *let-7*. LIN28b likely targets other molecules besides *let-7* that are involved in the intestinal cell-growth balancing act.

SCIENTIFIC PRESENTATION

New Frontiers in Intestinal Stem Cell Biology
Researchers in Dr. Rustgi's lab are now pursuing the discovery of other molecular targets of LIN28b besides *let-7* using new RNA sequencing techniques that allow the identification of targets of RNA binding proteins. Surprisingly, most of the targets discovered to date by Dr. Rustgi's lab participate in pathways related to metabolism and protein complexes at cell connections, presenting opportunities for new lines of research into the relationship between these pathways and disease states such as cancer. Dr. Rustgi and his colleagues plan to continue their search for new insights into the complex opposing forces underlying intestinal stem cell biology, RNA processing pathways, and diseases of the gastrointestinal tract.

INTESTINAL STEM CELL CONSORTIUM

In 2009, the NIDDK teamed up with the National Institute of Allergy and Infectious Diseases (NIAID) to establish the Intestinal Stem Cell Consortium. This Consortium brings together a group of investigators from research centers across the country to collaborate on a common mission: to advance understanding of stem cell biology in the intestinal epithelium and its role in organ development, homeostasis, regeneration, and disease, in order to provide a foundation for the development of new therapies for digestive diseases. The Consortium grew out of a March 2008 workshop hosted by the NIDDK on "Local Influences on Health and Repair of Intestinal Epithelium" and also aligns with multiple goals in the March 2009 research plan of the National Commission on Digestive Diseases. To date, Consortium support has resulted in the publication of 28 original scientific articles and counting. These research advances offer fresh insights into intestinal stem cell biology, as well as highlight new research methods and resources to facilitate future progress in this field.

In 2013, the NIDDK and NIAID announced an initiative to continue this productive Consortium. As part of the renewed Consortium, scientific exchange will be maximized and research accelerated through sharing of information, data, biomaterials, models, reagents, resources, and methods within the Consortium and with the larger research community. A coordinating center will facilitate the Consortium's activities and communication of research results, data, and methods amongst members and with the community. The Consortium will continue to support ongoing studies to isolate and characterize intestinal stem cells, compare stem cell populations, and determine how their activity is controlled. While the Consortium emphasizes research on stem cells of the small intestine, methods and research results stemming from these projects are expected to aid similar studies relevant to other areas of the gastrointestinal tract and other organs.

Additional information about research supported by the Intestinal Stem Cell Consortium can be found on its website at: http://iscc.coh.org/

PATIENT PROFILE

Nancy Norton
Living Hour to Hour with Fecal Incontinence


Nancy Norton

On many days, Nancy Norton can only plan her life hour by hour. When she was 35, while giving birth to her son, Nancy experienced a fourth degree perineal tear (a tear extending from the vagina to the rectum). The injury included damage to the anal sphincter muscles, which play a critical role in controlling bowel evacuations. As a result, for the past 28 years she has lived with fecal incontinence, which is the accidental passing of solid or liquid stool or mucus from the rectum.

Nancy's incontinence was revealed when, shortly after giving birth, she was hosting friends and family at her home in Milwaukee to see her new baby. "I was sitting in a chair, and I just stood up and had stool running down my leg," she recalls. Like many women who have experienced similar injuries, Nancy was caught completely off guard. Mortified and alarmed, she contacted her doctor immediately, telling herself, "I need to find out what's going on here."

When Nancy first contacted her health care provider, the response she received conveyed a practically dismissive tone. She was told there was a flu going around, and perhaps she just had an upset stomach. While Nancy felt her doctors were seeing her incontinence as little more than an inconvenience, she was starting to suspect that it was something more serious than the flu. When the condition did not improve, she underwent two rounds of surgery with the hope that repairing the injured tissue would be enough to resolve her incontinence. The first repair broke down, however, and the second repair was not successful. Nancy gradually began to realize that she might be facing a long battle with incontinence. "After waking up for months, hoping that this day was going to be the day when everything was going to work right," she remembers, "it starts to sink in that this is not changing." With no easy answers, Nancy felt stranded. "I was like a lot of other people, where it was a result of an obstetrical injury, and you just automatically assume that it can be fixed. But when the attempts to fix it don't work, then what do you do?"

"After waking up for months, hoping that this day was going to be the day when everything was going to work right," says Nancy, "it starts to sink in that this is not changing."

That time was especially challenging for Nancy because she had also just become a mother. "Here's this new little baby," she says, remembering those tough first days of parenthood, "and I was trying to be

PATIENT PROFILE

a mother while going back in the hospital for surgery. It was overwhelming." She faced juggling many major changes in her lifestyle at once: being a new parent, changing her diet, trying always to be near a bathroom, and bringing a bag of extra clothes and cleaning materials whenever she went out. "I was learning how to live with this new body, and being a new mom, and caring for the needs of my son," recalls Nancy. As a result of her experiences, she now understands the importance of not only addressing the medical issues associated with fecal incontinence, but also thinking about its effects on family and personal life.

Fecal Incontinence: A Hidden Condition

Nearly 18 million U.S. adults—about 1 in 12—have fecal incontinence, commonly referred to as bowel control problems. The high prevalence of this condition may seem surprising, but this is probably because most people who have fecal incontinence are reluctant to talk about it, and health care providers rarely screen for it, so it is significantly underdiagnosed. Besides feeling too embarrassed to discuss episodes of fecal incontinence, patients are also mired by the suspicion that their health care providers will not be able to help them, or they may just despondently accept the condition as a "normal" part of aging.

While fecal incontinence is more common in older adults, it can affect people of any age. Several factors can increase the risk of a bowel control problem, including: diarrhea; urgency, or the sensation of having very little time to get to the toilet for a bowel movement; a disease or injury that damages the nervous system; injury to the pelvic floor (the muscles, ligaments, and tissues that support the uterus, vagina, bladder, and rectum), which may occur during a difficult childbirth; and poor overall health from multiple chronic (long-lasting) illnesses. Other risk factors include depression, being physically inactive or overweight, or having type 2 diabetes.

Regardless of the cause, a serious case of fecal incontinence means a life that revolves around being near a bathroom, Nancy explains. This might mean only being able to make plans for the next hour or two at a time, which has the tendency to feel as if the incontinence is controlling a person's daily life. "Most people don't even think about the process of defecation, but you end up focusing almost 24/7 on controlling your bowels," she says. To make matters worse, accidental bowel movements carry such a social stigma that, rather than seeking help, many people with fecal incontinence painstakingly attempt to hide their symptoms with accessories like absorbent padding and deodorant sprays, or they try to manage the condition through changes in their diet that are not always healthy. They especially become very conscious of how much food and water they are taking in—sometimes to drastic levels. "A lot of incontinent people will think, 'If nothing goes in, nothing will come out,'" Nancy explains. "Well, that's not a healthy way to live. But it's those kinds of things that naturally go through your mind to try to control fecal incontinence."

"Most people don't even think about the process of defecation, but you end up focusing almost 24/7 on controlling your bowels," Nancy says.

Instead of leading a secret life, Nancy chose to keep looking for answers. After her surgical repairs failed to cure her incontinence, she set off to find doctors at some of the country's best medical institutions who may have other ideas for treatments. Some suggested more surgery, which Nancy was reluctant to try; others suggested a less familiar type of therapy called "biofeedback." Biofeedback, which teaches patients how to control bodily functions, is used for a number of conditions; for fecal incontinence, it is designed to educate people on the muscles that

control bowel evacuations. It improves the awareness of sensations in the lower intestine by using tools such as small inflatable balloons in the rectum, which assist in teaching people with fecal incontinence to coordinate the sensation of rectal filling with squeezing of the external sphincter muscle. Another aspect of biofeedback involves special sensors which measure bodily functions and deliver information to health care providers, who use the readings to help their patients make beneficial changes. Research sponsored by the NIDDK has provided encouraging results supporting biofeedback as an effective method for treating fecal incontinence.

Biofeedback helped Nancy regain some control over her daily life. "Biofeedback really helps you understand the physiology of how things work, about muscle groups and coordination of those muscle groups, so even though I am still incontinent, I feel like I have some kind of control," she says. It helped her learn how to be prepared and to think about what she needs to take with her during the course of the day. Even then, adjustment back to a daily routine required small steps. She would start by leaving her home for just a short period of time. She would ask herself, "could I be away from the house for an hour comfortably without being near a bathroom?" She forced herself to take a class at a university because it put her in a social situation where it would be difficult to be able to get up and leave. She made an effort to "get back out there and do things" that wouldn't result in her being overcome with anxiety, which can raise the sense of needing to be near a bathroom. To be out in public and have access to a restroom is not always easy, says Nancy, but "as an incontinent person, you learn how to navigate through life in a different way."

Advocating for Others

As a result of her experiences with fecal incontinence, her struggles with finding proper care, and her continuing journey through life with the condition, Nancy founded the International Foundation for Functional Gastrointestinal Disorders (IFFGD), a nonprofit education and research organization that helps people affected by gastrointestinal disorders. "I thought, I can't be the only one who is having these kinds of issues," she says. After IFFGD was established, "we had people calling us with every gastrointestinal condition you can think of because there was no other place for them to go." Since its start in 1991, the IFFGD has been a source of educational information, support, and assistance for people who suffer with functional gastrointestinal and motility disorders, including fecal incontinence and irritable bowel syndrome.

"I thought, I can't be the only one who is having these kinds of issues," says Nancy. After she established the International Foundation for Functional Gastrointestinal Disorders, she says, "we had people calling us with every gastrointestinal condition you can think of because there was no other place for them to go."

Even with a resource like the IFFGD, raising awareness of fecal incontinence is not an easy task, primarily because of the negative public perceptions of the condition. In a sense, fecal incontinence is a hidden disability, because the anxiety associated with it is an enormous burden, yet people tend to suffer in silence, or they rarely leave their homes. Travelling becomes an even bigger problem. Nancy knows "the anxiety of not being able to get out of your seat for the last half an hour [of a flight], and you just hope that everything is fine. Or checking into a hotel room early because you want to get into your own room and have your own bathroom." Even taking care of simple errands like going to the grocery store becomes an exercise in preparedness, as people with fecal incontinence

must be constantly aware of where the restrooms are. Even then, they must bring extra supplies, such as incontinence wipes, because public restrooms are ill-equipped to help someone clean themselves after an episode of incontinence.

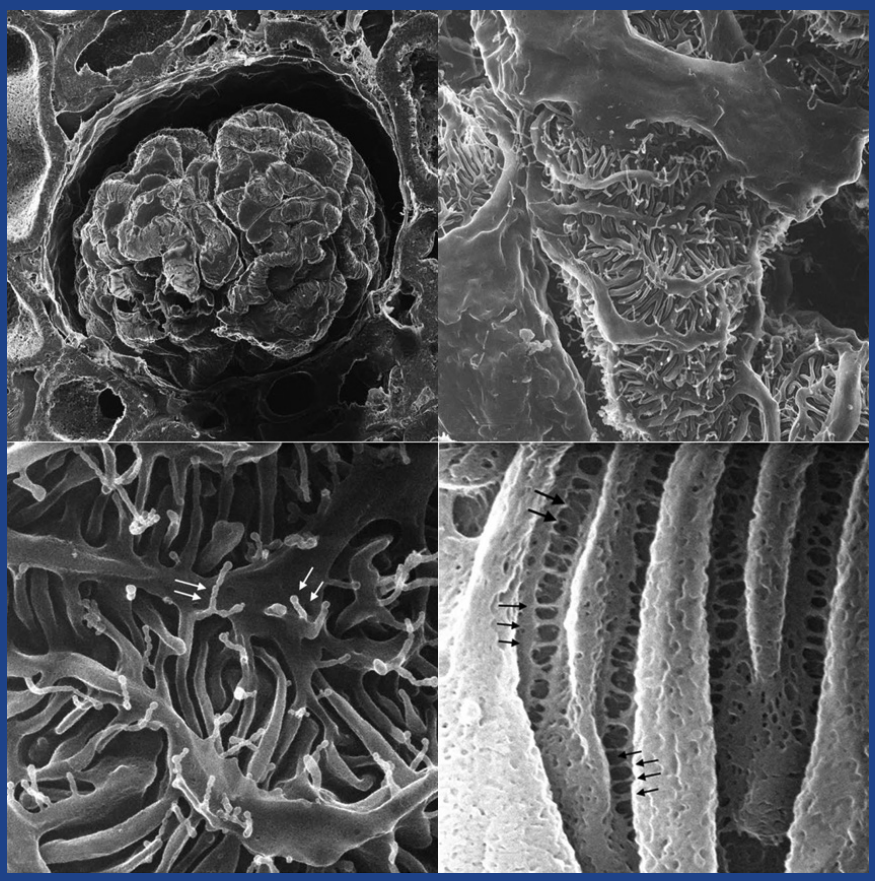
To be out in public and have access to a restroom is not always easy, says Nancy, but "as an incontinent person, you learn how to navigate through life in a different way."

The fear of having an accidental bowel movement in public can also be debilitating to someone's social life, especially if they are too embarrassed to explain why they cannot attend a gathering. Nancy is all too familiar with the questions that go through the mind of a person with fecal incontinence when asked to go out: "Do I feel comfortable enough to explain that I may not be able to make it? I might not be able to go to church, or sign up to volunteer for something; am I comfortable enough in explaining why I'm not there?" For the most part, says Nancy, people are understanding, but they do not necessarily comprehend the nature of her condition. People with fecal incontinence, she says, have to decide: "Are we going to venture out? How do we plan our day? What are we going to need? How are we going to handle [the incontinence]?" When accidents do happen, they can be psychologically crippling. "It's a very emotional experience when it happens, particularly in public," says Nancy about fecal incontinence. "It's enough to stop a person in his tracks, and then you don't want to go back out there again. You don't want to put yourself in that situation, because society is not always kind to people who are incontinent."

Hope Through Research and Awareness

Nancy believes that perception of fecal incontinence has improved slightly since her diagnosis almost 3 decades ago, but there is still much work to be done to raise awareness among medical caregivers and the public. Since 2011, the NIDDK has managed the Bowel Control Awareness Campaign, which provides current, science-based information about the symptoms, diagnosis, and treatment of fecal incontinence, including resources such as advice for talking to doctors and links to professional and voluntary organizations for help and support. In August of 2013, the NIDDK hosted a workshop entitled "Developing a Clinical Research Agenda for Fecal Incontinence," where a panel of experts discussed major issues in the diagnosis and treatment of fecal incontinence, and future research directions. There were also discussions about raising public awareness of fecal incontinence to make it easier for patients with the condition to come forward and seek treatment.

Nancy continues to live with fecal incontinence. But, as an advocate for millions of people who are affected by a largely secret condition that seizes control of their daily lives, she has shown the courage to confront negative perceptions and inspire hope in what many would see as a hopelessly distressing situation. She credits her biofeedback therapist and a strong network of people close to her as vital components to help her cope with incontinence. She says her husband, a co-founder of IFFGD, "has been by my side from the beginning, never wavering, always there to help me every day." She also has a group of supportive friends who understand her difficulties with social gatherings. "They're always saying things like, 'What can we all do that Nancy can do?' And that really means a lot to me. And I have been very thankful." She adds, "But this is something that is not going away, and there are a lot of people who really need help… [They are] living with fecal incontinence and are really doing the best they can to manage it."



Helium ion microscopy (HIM) uses a beam of helium ions to produce striking, high-magnification images that are difficult or impossible to capture with other types of microscopy. Sample preparation for HIM is relatively simple and allows researchers to view never before seen details of intact biological structures. Shown above are HIM images of rat kidney epithelium illustrating a basic kidney filtering unit, called a glomerulus. Glomeruli filter the blood, which is the first step in producing urine. The top left image shows a whole glomerulus, including the capillary bundle in the center. The other images are taken at increasing magnifications and show the glomerulus with the capillaries removed. The top right and bottom left image show two different magnifications of protrusions, called podocyte processes (white arrows), on the glomerulus' surface. The bottom right image was taken at yet higher magnification and shows the filtration slits on the podocyte processes (black arrows). As described in this chapter, researchers use imaging technologies such as HIM to study cell surface structures and membrane organization, which are the basic foundation for all organ function.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The overall goal of the NIDDK's research programs in these areas is to increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about two quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life-threatening condition.

It has been estimated that more than 20 million Americans have impaired kidney function—also called chronic kidney disease (CKD).[1] CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated.

CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2011, nearly 616,000 patients received treatment for ESRD: over 427,000 received either hemodialysis or peritoneal dialysis and over 185,000 were living with a kidney transplant. Minority populations, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic whites.[2] American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic whites. In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. The NIDDK's chronic renal diseases program supports

[1] *Levey AS, et al. Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2013 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2013.*

basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The Institute's Chronic Renal Insufficiency Cohort (CRIC) Study, one of the largest and longest ongoing studies of CKD epidemiology in the United States, has recently been extended for an additional five years. A major goal of this extension is to recruit an additional 1,500 people to the existing group of nearly 4,000 volunteers. These new participants and the additional time will allow researchers to collect more data and to explore and build upon findings compiled over the past 10 years, and examine in much greater detail the broad range of illnesses experienced by people with CKD. The NIDDK also supports studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, and urinary tract infections. As described in this chapter, NIDDK-supported research has identified a protein that functions to protect the urinary tract from bacterial infection. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS)—also known as IC/bladder pain syndrome (BPS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent) U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS.[3] Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with painful bladder syndrome.[4]

NIDDK-supported basic and clinical research on IC/PBS is focused on elucidating the causes of these conditions, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. These include the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research network, which supports research designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. To continue the Network's important efforts, the NIDDK recently issued multiple Requests for Applications (RFAs) to support the renewal and enhancement of this multi-center Network for a second five year funding cycle beginning in FY 2014. Other studies include the Boston Area Community Health Survey (BACH), which seeks to identify patterns and risk factors for a range of urological problems, and the Olmsted County (Minnesota) Study, which is studying lower urinary tract symptoms in men. As described later in this chapter, researchers recently used data from the BACH to look for possible relationships between consumption of certain beverages and lower urinary tract symptoms.

[3] *Berry SH, et al. J Urol 186: 540-544, 2011.*

[4] *Link CL, et al. J Urol 180: 599-606, 2008.*

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women.[5] Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes. To address this challenge, the NIDDK launched and recently expanded the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The Institute is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression in women, thereby improving health. In FY 2014, the NIDDK will hold a major multidisciplinary scientific symposium and pursue additional efforts focused on prevention of urinary incontinence and other lower urinary tract symptoms in women.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. NIDDK-supported research has recently identified a potential new approach to improve blood stem cell transplantation.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the NIDDK's hematology research program is the development of improved iron-chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases. As described later in this chapter, scientists report a potential new prevention or treatment approach for iron overload. Also later in this chapter, new research reveals how chemotherapy blunts blood stem cell renewal.

INSIGHTS INTO KIDNEY DISEASE

Deterioration in Heart Function Associated with Progression to Kidney Failure: The progression of chronic kidney disease (CKD) to kidney failure (end-stage renal disease or ESRD) is associated with less efficient pumping of blood by the heart. This decline in the "ejection fraction"—the amount of blood that leaves the heart with each contraction versus the amount that is left behind—may contribute to the increased risk of cardiovascular disease and death that is seen in patients who are undergoing dialysis. These findings come from the Chronic Renal Insufficiency Cohort (CRIC) Study, one of the largest and longest ongoing studies of CKD epidemiology in the United States.

In the current report, CRIC researchers focused on a subset of patients who had progressed from advanced CKD to ESRD over the course of the study, and who had undergone an echocardiogram—a test that produces a detailed moving image of a beating heart—both while they had advanced CKD and shortly after they had progressed to ESRD. These tests provided detailed "before" and "after" information about the patients' heart structure and function as their kidney function deteriorated.

Nearly three-quarters of patients with advanced CKD or ESRD have a condition termed left ventricular hypertrophy, which means that the main pumping chamber of their hearts is larger than normal because it must work harder to pump blood throughout the body. The researchers noted that there was no difference in

[5] *Urological Diseases in America, NIDDK, NIH Publication Number 12-7865, 2012.*

the degree of left ventricular hypertrophy as patients progressed from advanced CKD to ESRD. However, the average ejection fraction decreased slightly, but significantly, during the transition to ESRD. This was observed across all patients regardless of their age, race, diabetes status, or the type of dialysis (hemodialysis or peritoneal dialysis) they were receiving.

This is the first study to examine changes in heart structure and function in patients as they progress from CKD to ESRD. Future studies will explore the mechanisms responsible for the decline in ejection fraction that accompanies progression to ESRD.

Bansal N, Keane M, Delafontaine P, et al. A longitudinal study of left ventricular function and structure from CKD to ESRD: the CRIC study. Clin J Am Soc Nephrol 8: 355-362, 2013.

Tracking the Origins of Kidney Fibrosis: Researchers have used a series of genetically engineered mice to identify cellular sources of the fibrosis that follows kidney injury.

Fibrosis is a common final pathway for many diseases. Extensive kidney fibrosis, and the scar tissue that can sometimes arise from it, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure.

It is widely accepted that the source of collagen that causes fibrosis in the kidney is a type of cell called a myofibroblast. Previous research suggested that these cells might be derived from pericytes, a type of cell associated with blood vessels. In the new study, the scientists developed a number of different strains of mice in which they could visualize, track, and selectively eliminate specific subtypes of cells in the kidney. The goal was to identify the contribution of each cell subtype to the process of fibrosis.

The researchers confirmed that myofibroblasts are a significant contributor to kidney fibrosis. They identified two primary sources for these cells: about half of them result from conversion of existing precursor cells in the kidney, which then proliferate, while about one third arise from precursors produced in the bone marrow that travel to the kidney and convert to myofibroblasts but do not proliferate. (The remaining myofibroblasts were found to be derived from other sources.) The scientists also found evidence that pericytes were not a significant source of myofibroblasts.

Understanding the cellular and molecular mediators of kidney fibrosis is a high priority for scientists studying kidney disease. This finding is significant because it suggests that treatments for fibrosis that target myofibroblast proliferation may only be partially effective, because they have an impact on only about one-half of the myofibroblast population in the kidney. A better understanding of fibrosis, in general, could yield insights into how this process unfolds in other tissues and organs, potentially opening new avenues to therapy for a range of diseases.

LeBleu VS, Taduri G, O'Connell J, et al. Origin and function of myofibroblasts in kidney fibrosis. Nat Med 19: 1047-1053, 2013.

KIDNEY DISEASE AND GENETICS

Mutations May Lead to Both Kidney and Blood Diseases: A recently discovered set of gene mutations may be behind some cases of the serious blood disorder hemolytic uremic syndrome (HUS).

Hemolytic uremic syndrome is a condition that results from the premature destruction of red blood cells. These red blood cell fragments can clog the kidneys' filtering system and cause kidney failure. While many cases of HUS result from a bacterial infection, so-called atypical HUS (aHUS) arises from genetic or autoimmune factors. Researchers have now identified a set of novel mutations in the gene for diacylglycerol kinase epsilon (*DGKE*) that is associated with aHUS in nine unrelated families. The protein encoded by the *DGKE* gene is found in several cell types, including the blood vessels and filtering cells of the human kidney; thus, its disruption has the potential to have wide-ranging effects throughout the body.

People with the mutated *DGKE* genes typically develop aHUS before one year of age, have persistent high blood pressure, blood and protein in their urine, and are likely to develop chronic kidney disease as they age. The *DGKE* mutations identified in all nine families seemed to produce a non-functional, sometimes truncated DGKE protein, suggesting that the loss of DGKE function within cells might be responsible for these cases of aHUS. These findings implicate loss of DKGE function as a cause of a distinct subset of aHUS cases that may lead to severe kidney complications not usually seen in other forms of aHUS.

DGKE is the first gene implicated in aHUS that is not part of the complement system, a blood-borne part of the immune system. Currently, patients with aHUS are usually treated with drugs that inhibit the complement system, but this approach may be ineffective in people with *DGKE* mutations. For these individuals, kidney transplantation may represent a more effective treatment. These findings underscore the importance of correctly diagnosing patients whose aHUS arises from *DKGE* mutations and tailoring their treatment accordingly.

Lemaire M, Frémeaux-Bacchi V, Schaefer F, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nature Genet 45: 531-536, 2013.

Regulation of Cyst Growth in Polycystic Kidney Disease: Scientists have discovered a link between two proteins known to contribute to the most common form of polycystic kidney disease (PKD) and a cell-surface structure in a subset of kidney cells in mice.

PKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. For many people who have the more common form of the disease, autosomal dominant PKD (ADPKD), these cysts grow slowly over many years. Over time, they can profoundly enlarge the kidney and replace much of the organ's normal structure. This results in reduced kidney function that can potentially lead to kidney failure.

It is thought that cyst growth arises from improper growth signals in the kidney. Previous studies have shown that the removal of cilia, tiny, hair-like structures on the surface of some kidney cells, could produce cysts. Mutations in the proteins polycystin-1 and polycystin-2 have also been found to cause kidney cysts and cause ADPKD.

It is thought that cilia act to sense fluid flow in the kidney. The polycystins are present in the cilia, where they form a complex that has been hypothesized to aid in this flow sensing. To explore this relationship, researchers used a series of genetically engineered mice in which they could selectively delete polycystin-1, polycystin-2, and/or the cilia in the kidney. When they deleted polycystin-1 and/or -2, the mice developed kidney cysts. When the scientists next deleted the cilia in these mice, they found that cyst growth slowed dramatically. Interestingly, the severity of the cysts was related to the time interval between the loss of polycystin function and the loss of cilia: the longer the interval, the worse the cysts became before their growth slowed. This suggests that, under normal conditions, the polycystins may act as a brake on cyst-promoting signals arising from cilia. Loss of polycystin functions allows the cilia signal to proceed unabated, while deletion of cilia removes this stimulation.

These findings in mice have important implications for researchers' understanding of the molecular basis of kidney cysts. In people with ADPKD, most cysts are slow-growing, and people can live with the disease for decades before they develop symptoms. If similar mechanisms lead to kidney cyst development in people with ADPKD, then the design of strategies to inhibit the function of cilia that are associated with polycystins in these individuals before extensive kidney damage occurs could have profound implications for patient care.

Ma M, Tian X, Igarashi P, Pazour GJ, and Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet 45: 1004-1012, 2013.

GUT MICROBES AND BLOOD PRESSURE

New Function Identified for Odor Receptors: A family of complex cell-surface receptors, some of which were initially characterized as playing a role in detecting

odors in the nose, has since been found to be involved in the kidney's integration of signals from gut microbial metabolism and the regulation of blood pressure.

These specific cell-surface receptors are members of a family of proteins known as seven-transmembrane receptors, so-called because the proteins snake back and forth across the outer cell membrane seven times, forming a complex structure that helps the cell sense and respond to molecules that are outside of the cell. At least six members of the larger receptor family have been identified in the kidney, where they appear to influence the release of the hormone renin, which helps to regulate blood pressure by influencing the amount of fluid excreted by the kidneys. Two members of this receptor family that are present in the kidney—Olfr78, which was initially identified in the nose, and Gpr41, another seven-transmembrane receptor found in other cell types—are activated in response to short chain fatty acids produced by bacteria in the gut during digestion of fats or fiber.

To study the relationship between gut microbial-derived signals and kidney regulation of blood pressure, researchers administered the short chain fatty acid propionate to normal mice; they observed a rapid, but quickly reversible, drop in blood pressure. In subsequent studies, mice lacking the *Olfr78* gene were observed to have lower baseline blood pressure than normal mice, and proprionate further lowered blood pressure in these mice. However, proprionate administration *raised* the blood pressure of mice lacking the *Gpr41* gene, suggesting that the Gpr41 protein, when present, may respond to propionate by lowering blood pressure. Treatment with antibiotics, which disrupt normal gut function by killing intestinal bacteria, resulted in a significant increase in blood pressure in mice lacking the *Olfr78* gene, but had no effect on blood pressure in normal mice. Together, these results suggest that the presence of gut microbes producing proprionate may play a role in the modulation of blood pressure in mice. This effect appears to be mediated, at least in part, by *Olfr78* and *Gpr41*, which may work to balance one another to maintain normal blood pressure as proprionate levels in the blood fluctuate in response to gut microbial metabolism.

This discovery identifies a heretofore unknown connection between the gut, kidney, and cardiovascular systems. This connection may contribute to further understanding of high blood pressure and the future development of novel treatments.

Pluznick JL, Protzko RJ, Gevorgyan H, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci USA 110: 4410-4415, 2013.

KIDNEY DISEASE AND DIABETES

Test Predicts Outcomes in Dialysis Patients with Diabetes: In dialysis patients with diabetes, measuring another set of modified blood proteins may better predict the risk of death and cardiovascular disease (CVD) than the current standard test to assess blood glucose control used in the general diabetes population.

Diabetes is the leading cause of kidney failure, also termed end-stage renal disease (ESRD). Patients with ESRD have extremely poor survival rates, with fewer than 50 percent surviving three years after initiating dialysis. The leading cause of death in these individuals is CVD. The standard test used to determine blood glucose levels over the previous three months—hemoglobin A1c (HbA1c)—detects the fraction of circulating hemoglobin that has a glucose molecule attached to it. However, in people receiving hemodialysis—a process in which the blood is removed from the body, filtered, and returned—red blood cells, which contain hemoglobin, survive much less than three months. Thus, HbA1c can be misleading as a measure of long-term blood glucose control in these people. Alternatives to the HbA1c test exist, but they have not been evaluated with respect to long-term outcomes in dialysis patients.

To test other approaches to measuring blood sugar levels, and to evaluate their association with outcomes in dialysis patients, scientists examined blood samples from over 500 participants in a clinical trial; samples were taken at the time that the participants initiated dialysis and again at an average of five months later.

The researchers measured total glycated proteins, which represent all blood proteins that have a glucose molecule attached to them, and glycated albumin, which detects the fraction of the relatively common blood protein albumin that has undergone the same modification. They asked whether there was a correlation between elevated levels of these two values and increased risk of cardiovascular events, hospitalization due to sepsis (a serious bacterial infection), or death over an average follow-up period of three and a half years.

The analysis of the blood samples showed that elevated levels of overall glycated protein and the fraction of glycated albumin were associated with an increased risk of death from any cause, death from CVD, or time to first CVD event (for example, a non-fatal heart attack or stroke). In participants on dialysis, measurement of these markers may provide a more accurate representation of a given participant's blood glucose control, provide important information about risk of death and CVD, and could be useful for the management of diabetes in people who are on dialysis.

Shafi T, Sozio SM, Plantinga LC, et al. Serum fructosamine and glycated albumin and risk of mortality and clinical outcomes in hemodialysis patients. Diabetes Care 36: 1522-1533, 2013.

Shared Gene Networks Link Diabetic Kidney Disease in Humans, Mice: Scientists comparing networks of activated genes in the human and mouse kidney have identified several common patterns that may lead to the development of better mouse models of diabetic kidney disease.

While animal models have proven invaluable in biomedical research, their applicability to human disease can have limitations. Current mouse models of diabetic kidney disease most faithfully reproduce only the early stages of the disease; the disease does not progress in mice as it does in humans. To better understand these differences, researchers examined the patterns of genes that were turned on in three different mouse strains that have been used to model diabetic kidney disease and compared them with samples taken from people with diabetic kidney disease.

The researchers focused on genes in the glomerulus, the filtering unit within the kidney. Activation of certain signaling genes was observed in all comparison groups. Other genes were only activated in humans and one or two of the mouse models. This suggests that different mouse models may more closely replicate different aspects of human diabetic kidney disease.

Selection of the best mouse model to evaluate specific molecular pathways or potential treatments presents a significant research problem. The results of this study may help to unravel the complex network of gene activity that can lead to diabetic kidney disease. It may also facilitate future research endeavors by helping researchers select mouse models most applicable to a human disease process of interest, to focus on the biologic pathways most relevant to human disease, and to generate better mouse models of the disease for further study.

Hodgin JB, Nair V, Zhang H, et al. Identification of cross-species shared transcriptional networks of diabetic nephropathy in human and mouse glomeruli. Diabetes 62: 299-308, 2013.

VISUALIZING THE KIDNEY

A Closer Look at Kidney Structure: Researchers have unveiled a much closer, more detailed picture of the microscopic details of cells in the kidney.

Helium ion scanning microscopy is a new imaging technique that has been used to produce highly detailed pictures of inorganic materials. Now, this approach has been applied to biological specimens. In a recent publication, scientists presented images of multiple cell types and structures within the rat kidney. Kidney elements that were examined included the glomerulus and the branching appendages of podocytes, where blood is filtered; the proximal convoluted tubule and its brush border; and the collecting duct, which resorbs water and regulates blood pH.

Images generated through helium ion scanning microscopy are far more detailed than those produced

by scanning electron microscopy; the images in this study were captured at a resolution of approximately 1.4 nanometers (a nanometer is one-one billionth of a meter; for reference, the width of a human hair is approximately 80,000 to 100,000 nanometers).

This technological breakthrough in fine-scale visualization of cellular structures promises to allow more detailed studies of the cellular structure within tissues and facilitate scientists' understanding of cell architecture, organization, and the physical and spatial relationships that are involved in organ function.

Rice WL, Van Hoek AN, Păunescu TG, et al. High resolution helium ion scanning microscopy of the rat kidney. PLoS ONE 8: e57051, 2013.

FIBROSIS IN KIDNEY, BONE MARROW, AND UROLOGICAL DISEASES

The NIDDK is spearheading new efforts to learn more about fibrosis in kidney, bone marrow, and urologic diseases.

In October 2013, the NIDDK issued a solicitation, entitled "Novel Methods for Detection and Measurement of Organ Fibrosis in Kidney, Bone Marrow, and Urological Diseases," to encourage research. A consortium will be tasked with the development of novel methods for detection and measurement of organ fibrosis after acute or chronic injury in the kidney, bone marrow, prostate, or urinary tract. Specifically, the consortium will conduct translational research that focuses on development and validation of targeting probes, imaging technologies, or biomarkers to detect and measure pathologic fibrosis for molecular classification, risk stratification, and morphologic prediction as a step toward therapeutic prevention or reversal of fibrosis progression. It is anticipated that the consortium will begin its work in early 2015.

In January 2014, the Institute sponsored a scientific meeting, entitled "Targeting Fibrosis in Kidney, Bone Marrow, and Urological Diseases," to discuss detection and measurement of fibrosis in humans, in order to help inform future research efforts. The invited experts provided input regarding several issues including: the ability to differentiate between pathologic fibrosis and fibrosis in normal aging; whether fibrosis—as measured by imaging—correlates with organ dysfunction, recovery, and regression; and the novel biomarkers or technologies which should be pursued to detect and measure pathologic fibrosis.

"Fibrosis"—the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney—causing acute kidney failure—or from a slowly-progressing, chronic condition. Extensive kidney fibrosis, and the scar tissue that can sometimes arise, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure.

RESEARCH TOWARD PREVENTING URINARY TRACT INFECTIONS

Fending Off Infection in the Urinary Tract: Recent study has shown that ribonuclease 7 (RNase 7) contributes to defense of the human urinary tract against bacterial infection. The urinary tract is the body's drainage system for removing wastes and extra water. The system includes two kidneys, two ureters, a bladder, and a urethra. Despite its proximity to the anus, the urinary tract is usually sterile—but how it maintains its sterility is not well understood.

Building on previous research that demonstrated that RNase 7 is produced in human tissues (free of microscopic signs of disease or inflammation) of the bladder, ureters, and a specific part of the kidney called the collecting tubule, and is present in uninfected urine in sufficient quantity to kill bacteria, the same group of investigators has now reported the initial characterization of the antimicrobial features of RNase 7 in the human urinary tract during infection. Significantly more RNase 7 was detected in acutely inflamed kidney tissue compared to either non-inflamed or chronically inflamed kidney tissue. Consistent with findings included in the scientists' earlier study, non-inflamed collecting tubule produced RNase 7, as did acutely inflamed and chronically inflamed collecting tubule. However, in contrast to the non-inflamed condition, the kidney's proximal tubules produced RNase 7 in the setting of acute and chronic inflammation. The urine from children with bacterial infections contained significantly more RNase 7 compared to urine from uninfected children. RNase 7 was shown to be a potent, broad-spectrum antimicrobial agent against different types of bacteria (scientifically categorized as Gram-positive and Gram-negative), which are commonly found to cause urinary tract infections. RNase 7 exerts its antimicrobial activity by disrupting the bacterial cell membrane—making perforations such that the bacterium is no longer self-contained.

This study adds considerable knowledge to understanding how the urinary tract maintains sterility. Future studies that reveal the regulatory machinery involved in RNase 7 production or detail how this protein exerts its antimicrobial activity at the molecular level may help develop new therapeutic approaches to maintaining the sterility of the human urinary tract.

Spencer JD, Schwaderer AL, Wang H, et al. Ribonuclease 7, an antimicrobial peptide upregulated during infection, contributes to microbial defense of the human urinary tract. Kidney Int 83: 615-625, 2013.

"Survival of the Fitness" May Mean Multiple Reservoirs for Urinary Tract Infection-causing Bacteria: A new study suggests that the source of recurrent urinary tract infection (UTI) in women is more complex than previously thought, with potential implications for therapy. UTIs are common and occur more frequently in women, many of whom suffer repeated bouts of infection. UTIs are treatable with antibiotics, but the emergence of antibiotic-resistant microbes, combined with the personal and medical costs of care, makes finding better therapeutic strategies a priority. The primary culprit in UTIs is a bacterium called *Escherichia coli* (*E. coli*) that is found in the human gut. Most *E. coli* strains that live in the gut are harmless and actually play a number of beneficial roles, such as helping to prevent harmful bacteria from infecting the gut. Some *E. coli*, however, acquire the ability, through genetic changes, to infect the bladder, and these uropathogenic *E. coli* (UPEC) will cause a UTI if accidentally introduced to the urinary tract. Scientists have wondered whether genetic changes that enable *E. coli* to adapt to a new environment—in this case, the bladder—require a "trade-off" in which the bacteria are less fit to flourish in the gut. Answering this question could help in understanding UTIs and identifying the sites of bacterial reservoirs that could contribute to recurrent infection.

To test this idea, scientists studied 45 *E. coli* strains recovered from urine and fecal samples that were obtained from four women at each of three episodes of UTI. They sequenced the DNA and compared the genes, whole genome sequences, and growth and fitness profiles of the bacteria. Using this approach, the researchers uncovered two different sets of results. In two of the women, the dominant *E. coli* strains present

in the bladder and gut appeared to be genetically the same and did not change across the three UTI episodes. In the other two women, the dominant bacterial strains in the bladder and gut were also the same or very similar within each UTI episode, but changed between the first and third UTI episode. When the researchers compared the genetic profiles of strains from one woman whose bacteria changed between the first and third UTI, the results suggested that *E. coli* from the third episode would be better at infecting the bladder than *E. coli* from the first episode—and, thus, might be comparatively weaker in their ability to colonize the gut. However, when the scientists experimentally introduced the two strains into the bladder and gut of mice, they found that *E. coli* from the third episode grew more robustly than *E. coli* from the first episode not only in the mouse bladder, but also in the mouse gut—suggesting that greater infectivity in the bladder did not require a fitness trade off for growth in the gut. Previous research in rodent models has shown that some UPEC have the ability to "hide out" within bladder cells only to reemerge later—a possible source of recurrent infection. While this study focused on samples from a small number of women, the findings suggest that *E. coli* well-suited to cause UTIs may exist and flourish simultaneously in both the gut and the bladder, an aspect of UPEC that can be explored further as researchers consider how to design effective preventive and therapeutic strategies to combat recurrent UTIs.

Chen SL, Wu M, Henderson JP, et al. Genomic diversity and fitness of E. coli *strains recovered from the intestinal and urinary tracts of women with recurrent urinary tract infection. Sci Transl Med 5: 184ra60, 2013.*

BLADDER CONTROL AND OTHER LOWER URINARY TRACT SYMPTOMS

Feeling What You Drink: A new report suggests that limiting intake of caffeinated beverages may help stave off troublesome bladder and urinary symptoms. Many women and men live with symptoms affecting the lower urinary tract, such as frequent or urgent urination, needing to get up multiple times at night to urinate, and problems with voiding, such as a weak urinary stream or failure to empty the bladder completely. Dietary advice for people with these symptoms often includes avoiding caffeinated, carbonated, and citrus beverages because these drinks can irritate the bladder and therefore might also contribute to lower urinary tract symptoms; however, direct evidence for this association is limited. The Boston Area Community Health Survey (BACH) is a population-based study in white, Hispanic, and non-Hispanic black adults designed to assess prevalence and determinants of urological symptoms. Researchers analyzed dietary and symptom data collected from over 4,000 BACH study participants at both study entry (baseline) and about five years later to see if they could uncover any relationships between types and amounts of beverages (coffee, juice, and carbonated soda, including diet sodas and decaffeinated/caffeine free coffee and soda) and several lower urinary tract symptoms. They found that men reporting higher average caffeinated coffee or total caffeine consumption in the year prior to baseline—*e.g.*, more than two cups of coffee per day *versus* none—had a greater likelihood of symptom progression five years later, particularly symptoms of frequency and urgency. Drinking citrus juice, however, was associated with lower risk of symptom progression in men.

When they looked at changes in beverage consumption, the researchers found that women and men who increased their total coffee intake by at least two servings per day between baseline and the five year follow-up were more likely to have progression of urgency and frequency symptoms compared to those who had smaller changes in consumption; also, women who increased their total consumption of soda by at least two servings per day were more likely to have worsening of urgency symptoms. The researchers also examined short-term relationships between beverage intake and symptoms, and found that women and men who drank more than two cups of coffee or soda per day within the week prior to symptom assessment were more likely to have symptoms than those who did not; caffeinated diet soda appeared to affect women's symptoms at even lower consumption. While additional studies are needed to

verify these observations, the findings support current recommendations about limiting coffee and soda intake to help manage lower urinary tract symptoms and suggest that there are dietary components to be further explored for how they may cause urologic symptoms or, in the case of citrus juice consumption by men, possibly provide protection.

Maserejian NN, Wager CG, Giovannucci EL, Curto TM, McVary KT, McKinlay JB. Intake of caffeinated, carbonated, or citrus beverage types and development of lower urinary tract symptoms in men and women. Am J Epidemiol 177:1399-410, 2013.

Mouse Model Provides New Insight into Bladder Control: Recent research has identified a protein, β1-integrin, as having an essential role in bladder control in mice. The inner surface of the urinary tract is lined with epithelial tissue, or urothelium, which functions as a barrier to bacteria, environmental carcinogens, toxins, and the numerous and variable waste products in urine. The bladder is a balloon-shaped organ that stores and releases urine. The bladder muscle relaxes and stretches when it fills with urine, and it squeezes when it is time to urinate. As the bladder fills with urine, nerves carry signals about its change in shape and stretching—mechanosensory signals—to let the brain know when the bladder is full. Nerves also carry signals from the brain to tell the bladder when it is time to urinate. Improperly operating signals can lead to one of several conditions, including urinary frequency, urinary urgency, and urinary incontinence. Urinary frequency is an excessive number of urinations. Urinary urgency is the sudden, strong need to urinate immediately. Urinary incontinence (UI) is the unintentional leakage of urine.

Scientists have studied the ability of mice to maintain bladder control. A group of mice was genetically modified to no longer produce β1-integrin in the urothelium; a second group served as a normal population. β1-integrin, a member of a family of proteins called integrins, helps to anchor cells to the surrounding tissue. Surprisingly, the bladders of mice lacking the integrin were found to be normal in appearance. However, in contrast to normal mice, mice lacking β-1 integrin were found to have several abnormal bladder conditions that may reflect urinary incontinence, urinary frequency, and urinary urgency, and their bladders filled beyond normal limits before triggering urination. The study's findings in mice strongly suggest that loss of β1-integrin signaling in urothelium results in abnormal mechanosensory activity; information about the bladder's shape and level of fullness is not properly relayed or triggers improper responses. Future studies could explore whether people with urinary frequency, urinary urgency, and urinary incontinence also have abnormal integrin signaling in their urothelium. If the human bladder works similarly, then loss of normal urothelium mechanosensory signals due to disease or injury may lead to some forms of urinary frequency, urinary urgency, and urinary incontinence in people, and treatments based on this knowledge may follow.

Kanasaki K, Yu W, von Bodungen M, et al. Loss of β1-integrin from urothelium results in overactive bladder and incontinence in mice: a mechanosensory rather than structural phenotype. FASEB J 27: 1950-1961, 2013.

Clinical Trial Follow-Up Yields New Information on Surgery for Urinary Incontinence: A new report from a clinical trial comparing two surgeries to treat stress urinary incontinence (SUI) in women suggests that continued surveillance of outcomes is important in these patients. Women with SUI experience urine leakage under physical stress, such as coughing, laughing, sneezing, or lifting heavy objects. One treatment option for women with SUI is surgery to help prevent leakage; however, not much is known about how well different surgical approaches compare in terms of outcomes, both in the short and long term. The Trial Of Mid-Urethral Slings (TOMUS) study was conducted to compare the outcomes of two minimally invasive surgical sling procedures approved by the U.S. Food and Drug Administration to treat SUI in women. Both procedures use a synthetic mesh sling to support the urethra (the tube through which urine passes from the bladder to outside the body), thereby preventing urine leakage under stress; the procedures differ in how the mesh sling is inserted. In the trial, researchers randomly assigned nearly 600 women with SUI to either "retropubic" or "transobturator" midurethral

sling surgery, and then compared rates of treatment success at 12 and 24 months post-operatively. The trial used two measures of treatment success: surgery was considered an objective success if women had no leakage during a stress test and 24 hour pad test, and also had no retreatment for SUI; it was considered a subjective success if women did not self-report SUI symptoms, leakage, or a need for retreatment.

Previously, researchers reported the one-year follow-up results from TOMUS, which showed that both procedures help women achieve similar levels of dryness as measured by objective success measures; self-reported outcomes, although similar, did not meet the trial criteria for equivalence.

Now, the researchers have reported that, after 24 months and a modest drop in success rates for both procedures, the two procedures are no longer equivalent by either success measure; however, the researchers did not find clear enough differences between the success rates of the procedures to be able to recommend one procedure over another. Importantly, the TOMUS study also captured the risks and side effects of each type of surgery. For example, at 24 months, the group that underwent retropubic surgery had higher rates of voiding dysfunction requiring surgery, as well as more urinary tract infections; in contrast, women who had the transobturator surgery were more likely to experience neurological symptoms. Although the majority of adverse health events occurred in the first 12 months post-surgery, onset of one serious complication, mesh exposure through a surgical incision site in the vaginal wall, differed in its timing between the two surgeries: its occurrence was more likely within 12 months of retropubic surgery, but within the 13 to 24 month period after transobturator surgery. Still, participant satisfaction with both surgical procedures remained high, with accompanying improvement in symptom severity and quality of life measures.

Overall, these new findings from the TOMUS study highlight the evolution of outcomes and the continued occurrence of complications over time, and therefore suggest that continued follow up is important in women who have mid-urethral sling surgery for SUI.

Albo ME, Litman HJ, Richter HE, et al. Treatment success of retropubic and transobturator mid urethral slings at 24 months. J Urol 188: 2281-2287, 2012.

UNDERSTANDING AND TREATING HEMATOLOGICAL DISORDERS

Potential New Prevention or Treatment Approach to Iron Overload: Mini-hepcidins may provide benefit to people at risk for iron overload or people with iron overload. Hepcidin, a hormone produced by the liver, is the master regulator of iron balance in humans and other mammals. Hepcidin inhibits iron transport from cells in the intestine by binding to the iron channel, ferroportin, thereby reducing dietary iron absorption into the body. Insufficient levels of hepcidin cause or contribute to iron overload anemias such as hereditary hemochromatosis. Hepcidin deficiency is the cause of most cases of iron overload in people with hemochromatosis, a disorder in which the body absorbs too much iron and the extra iron builds up in organs. Strategies that increase the effective level of hepcidin might help ameliorate or prevent damage to organs, including the liver, heart, or pancreas for individuals with hemochromatosis and potentially other conditions marked by iron overload.

Building on previous research that demonstrated that miniature forms of hepcidin (fragments from one end of this hormone) could mimic hepcidin activity in mice, the same group of investigators synthesized a number of different versions of miniature hepcidins, tested these to identify an optimized "mini-hepcidin" called "PR65," and assessed its ability to prevent or treat iron overload in mice. Initially, PR65 was found to have superior potency and long-lasting action compared with natural hepcidin in normal laboratory mice.

Subsequent experiments utilized genetically engineered mice that no longer produced hepcidin. As hepcidin inhibits iron absorption, mice lacking hepcidin would be expected to absorb and store increased levels of iron. These mice were fed an iron-deficient diet for eight weeks and then placed on a diet high in iron for two weeks while simultaneously being given daily

injections of either PR65 or control injection (without PR65). Under these conditions, PR65 significantly reduced blood iron levels for up to 24 hours. In addition, PR65 prevented iron accumulation in the heart, liver, and blood of mice initially placed on an iron-depletion diet and then challenged with an iron-loading diet. In mice "pre-loaded" with iron using a standard diet, injection of PR65 daily for two weeks significantly reduced liver iron content by 20 percent but did not reduce iron content in the heart or blood.

Past investments in basic science research provided the foundation for this exciting study. Ongoing research is evaluating this and other promising compounds to effectively combat iron-related blood disorders.

Ramos E, Ruchala P, Goodnough JB, et al. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood 120: 3829-3836, 2012.

New Research Reveals How Chemotherapy Blunts Blood Cell Regeneration: A recent study conducted in mice has shown that chemotherapy damages nerves that regulate bone marrow niches responsible for making new blood cells (hematopoiesis). The hematopoietic niche of the bone marrow supports the survival and self-renewal of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs), yet prevents the ill-timed release of these cells into the circulation. When used to treat cancers such as non-Hodgkin's lymphoma and multiple myeloma, high-dose chemotherapy also destroys normal cells such as the HSCs and HPCs in the bone marrow. The result of chemotherapy, therefore, is a reduced hematopoietic reserve and function. However, the underlying cause remains unresolved.

This study shows for the first time that chemotherapy is toxic to the nerves that interact with (innervate) the hematopoietic niche. The experimental model assessed the ability of mice whose HSCs and HPCs have been destroyed (in this case, by irradiation) to restore their hematopoietic reserve and function under various conditions. Mice without HSCs and HPCs are likely to die unless transplanted with bone marrow cells from another normal animal. In one set of experiments, mice were or were not subjected to chemotherapy prior to irradiation and then transplanted with bone marrow cells. Compared to mice not subjected to chemotherapy, chemotherapy-treated mice were significantly more likely to die and contained fewer nerves innervating the hematopoietic niche.

To confirm that chemotherapy causes nerve toxicity in the bone marrow, the investigators studied the effect of a drug called 6-hydroxydopamine, which is known to selectively destroy "noradrenergic" nerves. Mice treated with 6-hydroxydopamine, a level of radiation that destroyed their HSCs and HPCs, and bone marrow cell transplantation showed a significant decrease in survival and delayed hematopoietic recovery compared to a group of mice not treated with the drug. Thus, this experimental model provides evidence that noradrenergic nerves are required for normal hematopoietic function. The loss of nerves contributes to the inability of the transplanted bone marrow cells to home to the bone marrow and take up residence in the hematopoietic niche.

The scientists next tested whether a known nerve protective agent such as 4-methylcatechol (4-MC) would mitigate the harmful effects of chemotherapy on hematopoietic nerves. In mice treated with chemotherapy and 4-MC, and subjected to lethal irradiation and bone marrow transplant, the number of hematopoietic nerves was significantly more than that found in mice not treated with 4-MC. In addition, the mice treated with 4-MC had improved survival. Thus the addition of 4-MC acts to maintain hematopoietic function by specifically protecting nerves which innervate the bone marrow. Overall, these results in mice may lead to future research in humans to explore ways to reduce nerve damage so as to improve blood cell regeneration after chemotherapy.

Lucas D, Scheiermann C, Chow A, et al. Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. Nat Med 19: 695-703, 2013.

Improving White Blood Cell Defense Against Bacteria: A recent study has shown that deletion of a protein in white blood cells improves their ability to eradicate infections with the bacteria *Staphylococcus*

aureus (*S. aureus*), although not the fungus *Aspergillus fumigatus* (*A. fumigatus*), in an animal model of chronic granulomatous disease (CGD).

CGD is a disorder that causes the immune system to malfunction, resulting in a form of immunodeficiency. Individuals with CGD have recurrent bacterial and fungal infections, and often have areas of inflammation (granulomas) in various tissues that can cause damage. The body's white blood cells normally eliminate bacteria via two modes of action—a non-oxidative mode of action that involves the use of specialized enzymes to attack and cleave proteins that are necessary for bacterial survival, and an oxidative mode involving chemically reactive molecules containing oxygen. It is the oxidative mode of defense that is defective in patients with CGD.

Building on their previous research findings showing that a protein called "olfactomedin-4" (Olfm4) hinders white blood cells' ability to eradicate bacteria, the researchers deleted the *Olfm4* gene in a mouse model of CGD and evaluated the impact of this deletion on host defense against *S. aureus* and *A. fumigatus*, both of which are common causes of infections in people with CGD. White blood cells obtained from mice lacking Olfm4 protein showed increased ability to kill *S. aureus* compared to white blood cells having Olfm4. Likewise, when mice lacking Olfm4 were exposed to *S. aureus* for six hours, they killed significantly more bacteria than mice with Olfm4.

Next, the investigators examined whether deletion of *Olfm4* in CGD mice enhanced host defense against infections of *S. aureus* and *A. fumigatus* as measured by survival over a two week testing period. When infected with *S. aureus*, all CGD mice with Olfm4 died within five days, while the majority (approximately 85 percent) of CGD mice without Olfm4 survived the two-week testing period. Notably, approximately 75 percent of normal mice with Olfm4 died within eight days. When infected with *A. fumigatus*, all CGD mice died within nine days, whether or not they had Olfm4. In contrast, all normal mice with or without Olfm4 survived the two week testing period following *A. fumigatus* infection.

These results show that *Olfm4* deletion can enhance host defense against *S. aureus*, but not *A. fumigatus*, in CGD mice. Future studies are needed to determine the role of Olfm4 in human white blood cells and could lead to the development of a therapeutic inhibitor of Olfm4 activity to boost human defense against infection.

Liu W, Yan M, Sugui JA et al. Olfm4 deletion enhances defense against Staphylococcus aureus *in chronic granulomatous disease. J Clin Invest 123: 3751-3755, 2013.*

Improving Blood Stem Cell Transplantation:
A recent study conducted in mice, baboons, and human volunteers has shown that a non-steroidal anti-inflammatory drug approved by the U.S. Food and Drug Administration (FDA), called meloxicam, significantly increased the number of blood (hematopoietic) stem cells (HSCs) and their descendent hematopoietic progenitor cells (HPCs) entering the circulation from the bone marrow, where they typically reside until needed. This finding sheds light on how the body responds to injury and has implications for blood cell transplantation. The hematopoietic niche of the bone marrow supports the survival and self-renewal of HSCs and HPCs, yet prevents the ill-timed release of these cells into the circulation. When used to treat cancers such as non-Hodgkin's lymphoma and multiple myeloma, high-dose chemotherapy also destroys normal cells such as the HSCs and HPCs in the bone marrow. To replenish the lost cells, HSCs and HPCs are routinely harvested from a donor's or patient's blood, and then transplanted back into the patient at the conclusion of the chemotherapy procedure to repopulate the bone marrow. The levels of HSCs and HPCs normally found in blood are very low, and strategies have been devised to mobilize HSCs and progenitor cells out of the bone marrow and into the circulation. The naturally occurring protein G-CSF often is used clinically to mobilize cells, but this strategy does not work for approximately 10 to 20 percent of individuals. Research continues in order to identify more effective strategies to mobilize cells out of the microenvironment of the marrow and into the circulation.

Building on their previous research findings, which showed that prostaglandin E2 enhanced HSC survival and homing to the bone marrow, researchers demonstrated that meloxicam treatment of mice greatly increased the numbers HSCs and HPCs in the circulation. Mice treated with a combination of G-CSF and meloxicam mobilized significantly more cells than those treated with either drug separately. Similar to findings in mice, meloxicam treatment of both baboons and healthy human volunteers increased HSCs and HPCs in the circulation. Additional experiments were conducted to determine how meloxicam exerts its biological effect. Previous research showed that prostaglandin E2 signals through one or more of the four E-prostanoid (EP1-4) receptors. Using genetically altered mouse strains each lacking one of the EP receptors, it was shown that mice lacking EP4 receptor had increased HSCs and HPCs in the circulation and meloxicam had no additional effect. These findings strongly suggest that meloxicam targets the prostaglandin E2/EP4 receptor signaling pathway.

Moreover, the researchers showed that mice deficient in osteopontin, a component of the HSC niche, mobilized HPCs but not HSCs when treated with meloxicam. This result demonstrates that meloxicam decreases osteopontin levels in the HSC niche, which then permits HSC, but not HPC, mobilization out of the marrow.

This study has several potentially important clinical implications. Meloxicam in combination with G-CSF may improve the success rates of blood stem cell transplantation by making it easier to obtain sufficient numbers of cells for transplant. Meloxicam is FDA-approved for use and therefore additional toxicology studies need not be conducted, saving both time and money. And, meloxicam has comparatively few side effects compared to other non-steroidal anti-inflammatory drugs.

Hoggatt J, Mohammad KS, Singh P, et al. Differential stem- and progenitor-cell trafficking by prostaglandin E2. Nature 495: 365-369, 2013.

Kidney Research National Dialogue

To inform Institute efforts, the NIDDK historically has solicited input from the research community, voluntary and professional organizations, and the public to identify research areas of particular opportunity or challenge. Recently, the NIDDK's Division of Kidney, Urologic, and Hematologic Diseases initiated an effort to seek input from stakeholders that would help the Institute identify and prioritize goals in kidney disease research. To do so, the Institute developed an interactive website: "The Kidney Research National Dialogue," or KRND.

The NIDDK used a Web-based interface to invite people who had an interest in kidney disease—scientists and clinicians, patients and their families, and others who wanted to contribute—to join in a national information exchange. Participants were asked to put forward their own ideas about the most exciting areas of kidney disease research, to read and comment on discussions from others about these ideas, and ultimately to prioritize these ideas by "voting" on the topics that they believed to be the most important. More than 1,600 participants posted approximately 300 ideas that had been broken out into 12 topic areas that cover the breadth of kidney physiology and disease, including: diabetic nephropathy, acute kidney injury (AKI), CKD, kidney biology, dialysis therapies, disease education, polycystic kidney disease, glomerular disease, pediatric kidney disease, and training.

The objective of the KRND was to engage a large number of people in a discussion of kidney disease, and to identify research strategies that would improve our understanding of normal kidney function and the mechanisms underlying kidney disease. The ideas emanating from these discussions are being distilled and published in a series of commentaries on different topics—making them available to the broader community interested in kidney disease research. The first commentaries were published in late 2013.

The KRND is a new, alternative approach in the evolution of the NIDDK's strategic planning process. The extended, open, Web-based dialogue used in the KRND allowed a large number of people to contribute to multiple simultaneous discussions that cut across diverse topic areas, and to do so at a time that was convenient for them. This Web-based approach was successful in compiling numerous posts and comments from a large, heterogeneous group of stakeholders whose expertise and interests spanned the kidney disease spectrum. The Institute is grateful for their input.

As of December 2013, three commentaries had been published (see below), another three have either been submitted or are in press, and the remaining ones were under development.

Rys-Sikora KE, Ketchum CJ, and Star RA, on behalf of the Kidney Research National Dialogue (KRND) Editorial Board. Kidney Research National Dialogue Overview and Commentary. Clin J Am Soc Nephrol 8: 1599 -1602, 2013.

Breyer MD, Coffman TM, Flessner MF, et al., on behalf of the Kidney Research National Dialogue (KRND). Diabetic Nephropathy: a National Dialogue. Clin J Am Soc Nephrol 8: 1603-1605, 2013.

Bonventre JV, Basile D, Liu KD, et al. on behalf of the Kidney Research National Dialogue (KRND). AKI: A Path Forward. Clin J Am Soc Nephrol 8: 1606-1608, 2013.

SCIENTIFIC PRESENTATION

Urothelium and Human Disease

Dr. Mark L. Zeidel

Dr. Mark L. Zeidel is the Herman Ludwig Blumgart Professor of Medicine at Harvard Medical School, and Physician-in-Chief and Chairman of the Department of Medicine at the Beth Israel Deaconess Medical Center. He is a widely recognized scientist, clinician and teacher, known for his research in the area of epithelial biology and water transport. Dr. Zeidel has been recognized by his peers for numerous accomplishments, including elected membership in the American Society of Clinical Investigation and the Association of American Physicians. A graduate of Yale College, he earned his M.D. from Columbia University College of Physicians and Surgeons. He trained in internal medicine and nephrology at Brigham and Women's Hospital, and was chief of the renal section of the West Roxbury VA, and Assistant Professor of Medicine at Harvard. He then moved to the University of Pittsburgh School of Medicine in 1993, where he served as chief of the renal and electrolyte division of the Department of Medicine, and then as the Jack D. Myers Professor and Chair of the Department of Medicine. He has been an NIDDK-supported researcher for the past 24 years. At the September 2013 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Zeidel presented a lecture on the role of the bladder urothelium in urological diseases.

To maintain a constant internal environment, or homeostasis, all organisms must regulate the flow of water across their membranes. The ability of water to flow through membranes varies greatly—by up to 4 orders of magnitude—and this process enables such necessities as preservation of normal blood composition. One example of abnormal water flow is when kidneys fail to reabsorb water, causing diabetes insipidus, which is characterized by frequent urination and excessive thirst (but normal blood sugar levels, unlike more common forms of diabetes). Dr. Zeidel's research group has led efforts to understand how specialized cells regulate ability of water and other molecules to flow through membranes; specifically how the urothelium—the lining of the urinary tract, including the bladder—maintains its homeostasis, and to gain insight into the diseases which affect the urothelium.

The Urothelium

The lower urinary tract is primarily made up of the ureter (the tube that connects the kidney to the bladder), bladder, and urethra (tube that carries urine from the bladder to the outside). Lining the lower urinary tract is tissue called the urothelium, which functions as a barrier to bacteria, environmental carcinogens, toxins, and the highly variable waste products in urine. The urothelium consists of three cell layers called the umbrella, intermediate, and basal cells. It is the outer (apical) surface of the umbrella cell which comes into direct contact with urine.

The urothelium's umbrella cell apical membrane creates a barrier that prevents water, urea, ammonia, and carbon dioxide from leaving the urine and entering the bladder tissue. To accomplish this, the umbrella cell apical membrane employs two approaches. First, membranes contain fat molecules (lipids) that are tightly packed into the bilayers of the membrane, and can further increase barrier function when a certain type of fat, cholesterol, is present. Second, the apical

surface of these cells contains plaques that cover the majority of the membrane surface of the bladder. The plaques consist of an ordered arrangement of proteins called uroplakins, which also contribute to the barrier function of the umbrella cell apical membrane.

In studies using mice that no longer produce uroplakins in their bladders, Dr. Zeidel, working with Dr. Tung-Tien Sun, of New York University School of Medicine, and colleagues showed a large increase in apical membrane water transport (permeability). This finding confirms that uroplakins contribute to barrier function of the umbrella cell apical membrane. In addition, Dr. Zeidel and colleagues have shown the importance of the umbrella cell layer to regulate permeability using an animal model of urothelium injury. Using a chemical called protamine sulfate which strips the umbrella cell layer off the urothelium, it was shown that removal of the umbrella cell leads to increased water and urea transport. Of note, the barrier function is rapidly restored within 3-5 days in the animal model.

The bladder is a very flexible, balloon-shaped organ that stores and releases urine. The bladder muscle relaxes and stretches when it fills with urine, and it contracts when it's time to urinate. As the bladder fills with urine, nerves carry signals about changes in bladder shape and stretching, *i.e.*, mechanosensory signals, to let the brain know when the bladder is full. Nerves also carry signals from the brain to tell the bladder when it's time to urinate. Thus, the apical membrane of the umbrella cell needs to be very flexible as the urothelium transitions from a "globular" structure when the bladder is empty to a "flagstone" shape when the bladder is full.

In studies conducted in collaboration with Dr. Jeffrey Fredberg at the Harvard School of Public Health, Dr. Zeidel has shown that the umbrella cell's apical membrane is one of the most flexible membranes known. To measure flexibility, magnetic beads were attached to the apical surface of urothelium membrane and subjected to magnetic fields, and their movement was captured using video microscopy. Both urothelium and red blood cells were found to be very responsive to the magnetic field. Moreover, in studies using mice that no longer produce uroplakins in their bladders, the urothelium was determined to be more rigid—losing a significant portion of its flexibility.

Also contributing to the flexibility of the urothelium is the uneven distribution of actin. One of the most common proteins found in mammals, actin has many functions, including its role in maintaining the cell's proper shape. Dr. Zeidel and colleagues found no detectable actin in the umbrella cell's apical membrane in contrast to a different part of the umbrella cell membrane, called the "basolateral" membrane, which comes into contact with adjoining cells. This lack of actin in the apical portion of the membrane is thought to allow for more flexibility as the bladder expands and contracts.

Insights into the Role of the Urothelium in Disease

Dr. Zeidel then described several experiments conducted by Dr. Warren Hill and his collaborators in the Department of Medicine at the Beth Israel Deaconess Medical Center, which were designed to illuminate whether the urothelium "communicates" with the nervous system to regulate bladder control. The scientists compared the ability of two groups of mice to maintain bladder control. One group of mice was genetically modified to no longer produce β1-integrin in the urothelium and the other group of mice served as a normal population. β1-integrin, a member of the integrin family of proteins, helps to anchor cells to the surrounding tissue. Surprisingly, the bladders of mice lacking the integrin were found to be normal

in appearance. However, in contrast to normal mice, mice lacking β1-integrin were found to have several abnormal bladder conditions that may reflect urinary incontinence, urinary frequency, and urinary urgency, and their bladders filled beyond normal limits before triggering urination. Urinary incontinence is the unintentional leakage of urine. Urinary frequency is an excessive number of urinations. Urinary urgency is the sudden, strong need to urinate immediately. Thus, the bladder urothelium senses bladder fullness and communicates this information through the β1-integrin to nerves that connect to the bladder.

An acute urinary tract infection begins when bacteria attach to the bladder urothelium. This provokes a defense response in the infected individual, including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the bladder of bacteria. Dr. Zeidel showed images, published by Dr. Scott J. Hultgren and colleagues of Washington University in St. Louis, of bacteria attaching to mouse bladder urothelium. The bacteria use adhesive structures called "type 1 pili" which serve to tether the bacteria to the apical surface of the umbrella cell. Once attached, the umbrella cell "engulfs" the bacteria and this sets in motion a host immune response to eliminate the infected cell.

Urothelium and Human Disease

Human diseases and disorders of the urothelium include cystitis, lower urinary tract syndrome (LUTS), and urothelial cancer. Cystitis, LUTS, and urothelial cancer are debilitating, burdensome, and costly conditions and in the case of urothelial cancer can be life-threatening.

Cystitis is the inflammation of the bladder and can be caused by bacterial infection or chemical exposure. Interstitial cystitis/painful bladder syndrome (IC/PBS) or bladder pain syndrome is one of several conditions that causes bladder pain and a need to urinate frequently and urgently. Both men and women can acquire IC/PBS, though twice as many women are affected as men. It can occur at any age, but it is most common in middle age.

LUTS is a syndrome featuring symptoms which occur during urine storage, voiding, or post-voiding. The symptoms include urine leakage, frequent and/or painful urination, sudden and/or strong urges to urinate, problems starting a urine stream, and/or problems emptying the bladder completely. Dr. Zeidel presented data obtained from the Epidemiology of LUTS (EpiLUTS) study, which was a large population study of the prevalence of LUTS in women and men over 40 years old. Although men and women both report having symptoms such as leakage and weak stream, the study findings indicate that LUTS impacts both men and women in very different but nonetheless very bothersome ways.

Dr. Zeidel suggested that several different abnormal mechanisms could be responsible for LUTS, including those targeting the urothelium such as barrier failure, inadequate repair, and poor sensing. In addition, the bladder wall's detrusor muscle may be deformed or in spasm. Due to the potential numerous causes of LUTS, therapies to address each of these have been developed and range from limiting fluid intake, to botulinum toxin injections to surgery. Dr. Zeidel commented that one need only go to a grocery store to find that the availability of diapers targeting adults now rivals that for infants. Drawing from his clinical expertise, Dr. Zeidel offered the following observations regarding LUTS. First, LUTS is extremely common and becomes more severe with aging. Second, the symptoms and their severity vary enormously between individual patients, and environmental elements play

a pivotal role. And third, the wide array of therapies, aimed at some but not all of the elements of normal voiding, indicate that we know little about the mechanisms of LUTS in individual patients.

Current and Future Research Directions

Dr. Zeidel's current research efforts include the development of two new mouse lines: one line that has a defined genetic background and the other line having a more mixed, or heterozygous genetic background (*e.g.*, similar to human genetics). These new laboratory resources will then be used to define the genetic contribution in mice displaying LUTS, particularly in older mice, which will increase our understanding of these symptoms, foster the design of new treatments, and ultimately pave the way to test prevention strategies.

STORY OF DISCOVERY

IgA Nephropathy—Shedding Light on a Form of Kidney Disease

Immunoglobulin A nephropathy (IgAN) is a kidney disorder that occurs when a complex of two immune system proteins that helps the body fight infections settles in the kidneys, eventually disrupting kidney function. While the mechanisms underlying this condition have remained mysterious, recent NIDDK-supported discoveries have provided important insights into molecular mechanisms that play an important role in this disease.

What Is IgAN?

IgAN is a caused by the abnormal deposition of immune system proteins in the small blood vessels within the kidneys. These proteins, which are types of antibodies called IgA and IgG, normally help the body fight infections, but when they form abnormal complexes that settle in the kidneys, serious problems can result. As these protein complexes accumulate over many years, they can lead to inflammation and scarring of the kidneys' filtering units, or glomeruli. This damage, along with the accompanying development of fibrotic scar tissue, can impair the kidneys' ability to effectively filter waste products and excess fluids and salts from the blood, and result in the leakage of blood and sometimes protein into the urine. Unchecked, this process can lead to a decline in kidney function or to kidney failure.

Because these proteins are part of the immune system, IgAN is considered to be an "autoimmune" disease. In conditions such as this, the body's defense mechanism that is designed to protect against outside threats, such as bacteria and viruses, somehow turns inward, attacking the body's own cells or molecules.

As is the case with many forms of chronic kidney disease, IgAN is often a "silent" condition. Many people with IgAN will not show symptoms for years, or perhaps decades. Unfortunately, about 25 percent of adults with IgAN will develop kidney failure, also called end-stage renal disease, requiring dialysis or a kidney transplant to live.[1]

IgAN can occur at any age, though it is less common in the very young and the very old. It is more likely to occur in men than in women. It is more common among Caucasians and Asians, and less common among African Americans.

Currently, there is no treatment that specifically addresses the disease mechanism that underlies IgAN. In formulating treatment strategies, physicians aim to ameliorate the consequences of the disease, using approaches that reduce tissue scarring in the kidney and controlling blood pressure (which, when elevated, can also result in kidney damage). However, recent discoveries have opened a window through which researchers are able to glimpse hints of the disease's underlying cause, providing hope for those seeking new treatments, whether they are patients or investigators.

[1] *IgA Nephropathy. National Kidney and Urologic Diseases Information Clearinghouse. Feb 2008. Pub. No. 08-4571.*

STORY OF DISCOVERY

History and Pathogenesis of IgAN

IgAN was first identified as a distinct clinical diagnosis in 1968, based on the accumulation in the glomeruli of the proteins IgA and IgG. IgAN remains a diagnosis that is characterized by the identification of deposits of these proteins in kidney biopsy tissue.

IgA, IgG, and Immune Complexes

Research results published in the early- to mid-1990s suggested that patients with IgAN had IgA antibodies that were deficient in a sugar called galactose. Specifically, researchers showed that IgA from the blood of patients with IgAN is deficient in galactose and that this deficiency occurs in a specific part of the antibody called the hinge region. The deficiency of galactose in the hinge region of IgA unmasks the presence of GalNAc (a sugar derivative of galactose), which is subsequently recognized by naturally occurring IgG and IgA antibodies in the circulation. Hence, an autoimmune condition is established when normal IgG and IgA antibodies now recognize the aberrant IgA as foreign and form immune complexes. In a study of European patients with IgAN, researchers found that increased blood levels of both normal IgA and IgG paralleled the severity of disease progression. In addition, patients with the highest levels of IgG against the aberrant IgA antibody at the time of diagnosis had the highest risk of kidney failure. In a similar study conducted in a Chinese population with IgAN, elevated blood levels of aberrant IgA were found to be associated with poor prognosis.

To understand and explore the mechanism(s) that lead to the formation of galactose-deficient IgA associated with IgAN, cell lines from circulating IgA1-producing B lymphocytes derived from patients with IgAN were established in the laboratory. The cell lines were shown to produce galactose-deficient IgA and allowed for the identification of the specific step in the biological pathway responsible for the addition of galactose to the IgA antibody. Thus, these cell lines may be a valuable resource in the development of new therapeutic strategies for IgAN. As IgAN disease onset and progression often coincides with an upper respiratory tract infection, the researchers evaluated immune-system regulatory molecules called cytokines for their ability to add sugar molecules such as galactose to IgA using the above mentioned cell lines. Cytokines are released from immune cells such as monocytes and macrophages. The study findings indicated that the cytokines interleukin-6 and (to a lesser extent) interleukin-4 significantly worsened galactose deficiency of IgA via the coordinated regulation of key enzymes.

Further research investigating the IgG antibody from IgAN patients using a cell culture approach revealed a change in the amino acid structure of the antibody; the IgG antibody has an “alanine” to “serine” amino acid substitution in the part of the antibody important for binding to its target—the aberrant IgA. In a proof of principle experiment, the investigators genetically engineered the serine-substituted IgG to contain the normal alanine amino acid and assessed its ability to bind aberrant IgA. The alanine-containing IgG was determined to have dramatically less binding capacity for aberrant IgA.

IgA Deposits

Researchers have shown that immune complexes containing aberrant IgA specifically associated with human mesangial cells more efficiently in laboratory experiments than did aberrant IgA alone. Mesangial cells are located within the central portion of the glomerulus and provide structural support. In addition, a greater amount of circulating immune complexes from a patient with IgAN bound to mesangial cells than did immune complexes from a healthy volunteer.

STORY OF DISCOVERY

Because mesangial cells are critical for kidney glomerular function, investigators systematically evaluated kidney biopsy tissues from patients with IgAN to begin to discover the molecular mechanisms that take place when IgA deposits in the mesangium. Previous research showed that many tissues and cell types use the "MAPK/ERK" signaling pathway in processes such as survival, inflammatory responses, and cell growth control, and investigators sought to determine whether this pathway was involved when IgA deposits in the mesangium. The study showed that the MAPK/ERK signaling pathway was activated in the mesangium of patients with IgAN having significant loss of kidney function. In contrast, kidney biopsy tissues from IgAN patients having better kidney function did not show activation of the MAPK/ERK signaling pathway.

Genetics of Disease

Researchers measured aberrant IgA levels in patients with IgAN, their relatives, and other volunteers without the disease. High levels of aberrant IgA were detected in blood from patients with IgAN compared to controls. Somewhat surprisingly, approximately half of the family members of IgAN patients also had elevated levels of aberrant IgA but did not display disease symptoms. The study results suggest that the defect in sugar addition to IgA antibodies is an inherited trait, but that additional factors—either genetic or environmental—are required for kidney disease to develop. A genome-wide association study of people of Chinese and European ancestry with IgAN has identified several regions of the genome (loci) associated with this disease. Genome-wide association studies involve rapidly scanning markers across the genome to find genetic variations associated with a particular disease. Scientists conducting these types of studies analyze genetic differences between people with a particular disease and healthy people.

Of the five loci contributing to significant risk for IgAN, one localized to the major histocompatibility complex (MHC)—a cluster of genes that play an important role in the immune system. MHC determines compatibility of donors for organ transplant as well as one's susceptibility to an autoimmune disease via crossreacting immunization.

The other loci contain genes associated with the immune response, leading to models of the disease as a multi-step process. Producing high levels of aberrant IgA can lead to the disease in individuals who are genetically predisposed to develop kidney injury due to an overactive or aberrant immune or inflammatory response.

Looking Forward

Despite recent progress toward discovering the molecular underpinnings of this multifaceted disease, an effective treatment for IgAN remains elusive. The NIDDK continues its robust support of basic research into normal kidney function and clinical research into the diseases that impair normal kidney function at the cellular and molecular levels. For example, the Institute established the "Cure Glomerulopathy Network" (CureGN) consortium in 2013 to support translational and clinical research that promotes therapeutic development for primary glomerular diseases, including IgAN (*https://curegn.org*). Research studies of families in which IgAN is prevalent continue in order to identify and understand the genes and genetic factors that influence disease onset and progression.

Through multiple avenues of research, the NIDDK aims to advance progress toward the development of new intervention strategies.

Funding Trends and Support of Core Values

NIDDK's core values emphasize maintaining a strong investigator initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials, and continuing strong support of training and career development programs, consistent with the vision of NIDDK Director, Dr. Griffin P. Rodgers (see Director's Message).

At NIDDK's May 2012 Advisory Council meeting, NIDDK's Deputy Director, Dr. Gregory Germino, highlighted these values and reviewed NIDDK's resource focus on areas supporting the core values.

Following that presentation, NIDDK generated additional data on application and funding trends to help our research community understand application and funding dynamics over recent years and demonstrate NIDDK's commitment to research and programs associated with NIDDK's core values and posted these data on the NIDDK website (*www.niddk.nih.gov*, in the "Funded Grants and Grant History" section). The data on the following pages were more recently updated to show trends through Fiscal Year 2013. NIDDK will continue to update these charts as new data become available.

THE NIDDK FUNDING OUTCOMES FOR FISCAL YEAR (FY) 2013 AND HISTORICAL APPLICATION AND FUNDING TRENDS

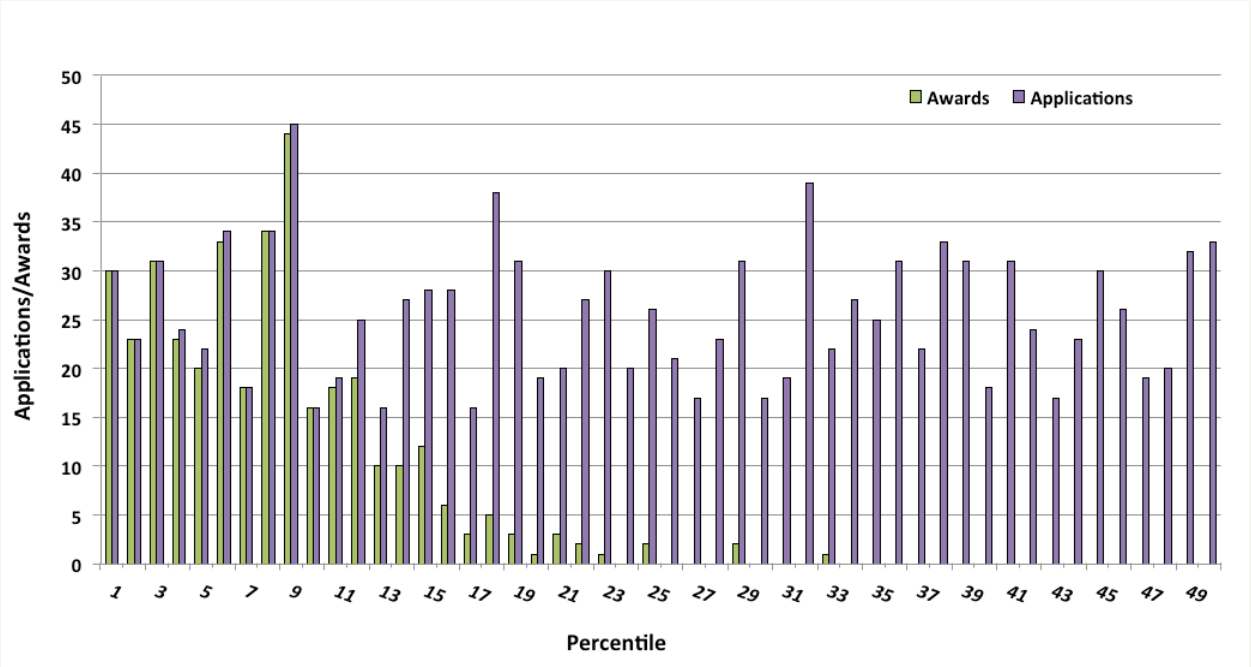
The data in all charts exclude initiatives (*i.e.*, RFAs), grants funded using the type 1 diabetes special appropriation and funds appropriated through the American Recovery and Reinvestment Act (ARRA).

GRANT ACTIVITIES

Code	Title	Code	Title
K01	Research Scientist Development Award Research and Training	R13	Conference
K08	Clinical Investigator Award (CIA)	R15	Academic Research Enhancement Award (AREA)
K23	Mentored Patient-oriented Research Career Development Award	R18	Research Demonstration and Dissemination Project
K24	Midcareer Investigator Award in Patient-oriented Research	R21	Exploratory/Developmental Grant
K25	Mentored Quantitative Research Career Development Award	R24	Resource-related Research Project
K99	Career Transition Award	R34	Planning Grant
P01	Research Program Project	R37	Method to Extend Research in Time (MERIT) Award
R00	Research Transition Award	SBIR/STTR	Small Business Innovation Research Grant/ Small Business Technology Transfer Grant
R01	Research Project	T32	Institutional National Research Service Award
R03	Small Research Grant		

FIGURE 1

FIGURE 1: NUMBER OF NIDDK COMPETING R01 APPLICATIONS SCORING WITHIN THE TOP 50TH PERCENTILE AND NUMBER OF NIDDK COMPETING R01 APPLICATIONS FUNDED IN FY 2013

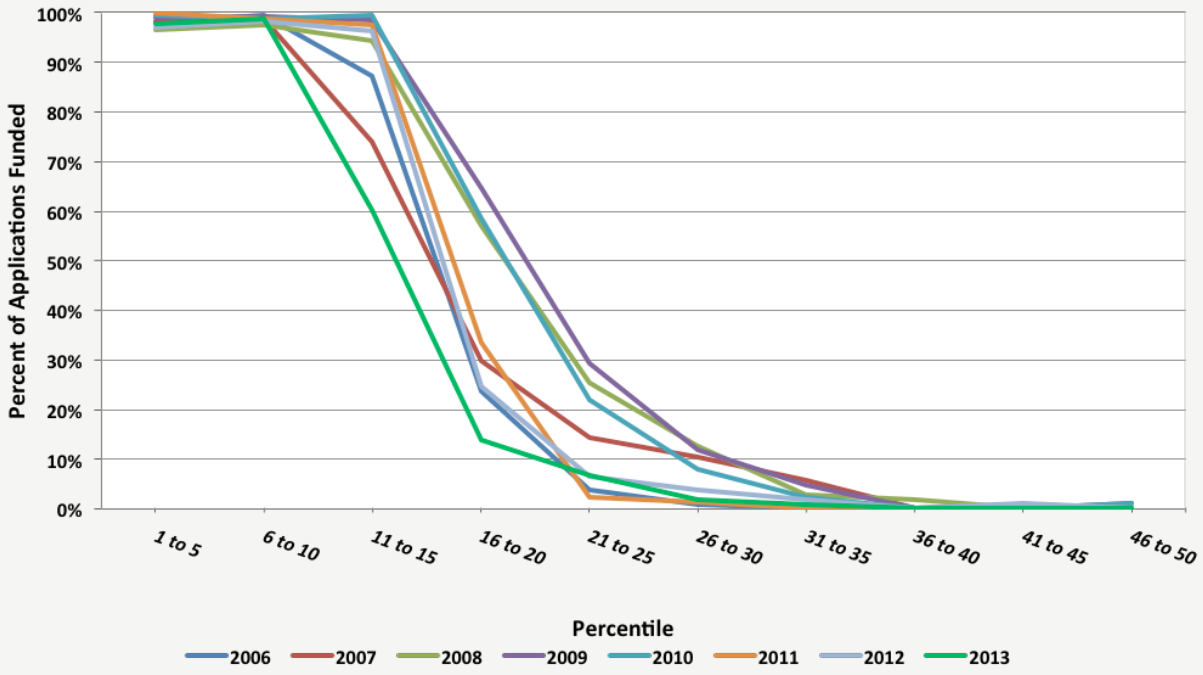


"Applications" shown in the chart above include all applications that scored 50th percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50th percentile are not shown (45 percent [n=1,064] of the applications received were unscored or scored above the 50th percentile). No unscored applications were funded in FY 2013.

The NIDDK nominal payline in FY 2013 was the 11th percentile for established investigators and the 16th percentile for Early Stage New Investigators. These data show that NIDDK closely adheres to its payline, but does exercise some programmatic discretion to reach for a limited number of especially innovative or programmatically important applications.

FIGURE 2

FIGURE 2: NIDDK COMPETING R01 APPLICATION FUNDING CURVES FOR FY 2006-2013



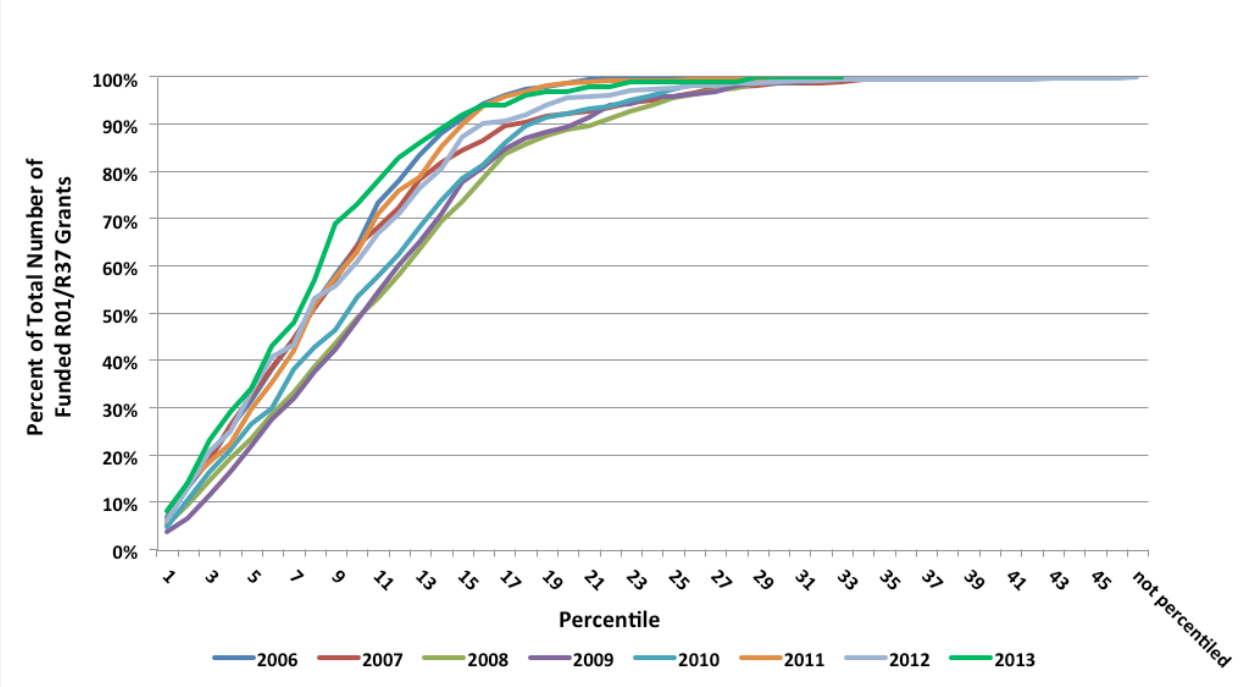
To generate the data for Figure 2, applications were placed into "percentile bins" as follows: Bin 1-5 includes all applications with percentile scores from 0.1 to 5.0, Bin 6-10 includes applications with percentile scores from 5.1 to 10.0, *etc*. Only applications that scored 50th percentile or better were included in the analysis.

The data demonstrate steep deflections in the percentage of applications funded at the nominal payline for each year. Paylines for the years included in Figure 2 are shown in the table to the right.

Fiscal Year	General Payline	New Investigator*/ Early Stage Investigator** Payline
2006	14	16*
2007	13	15*
2008	17	19*
2009	17	19*
2010	17	19*
2011	15	17*
2012	13	18**
2013	11	16**

FIGURE 3

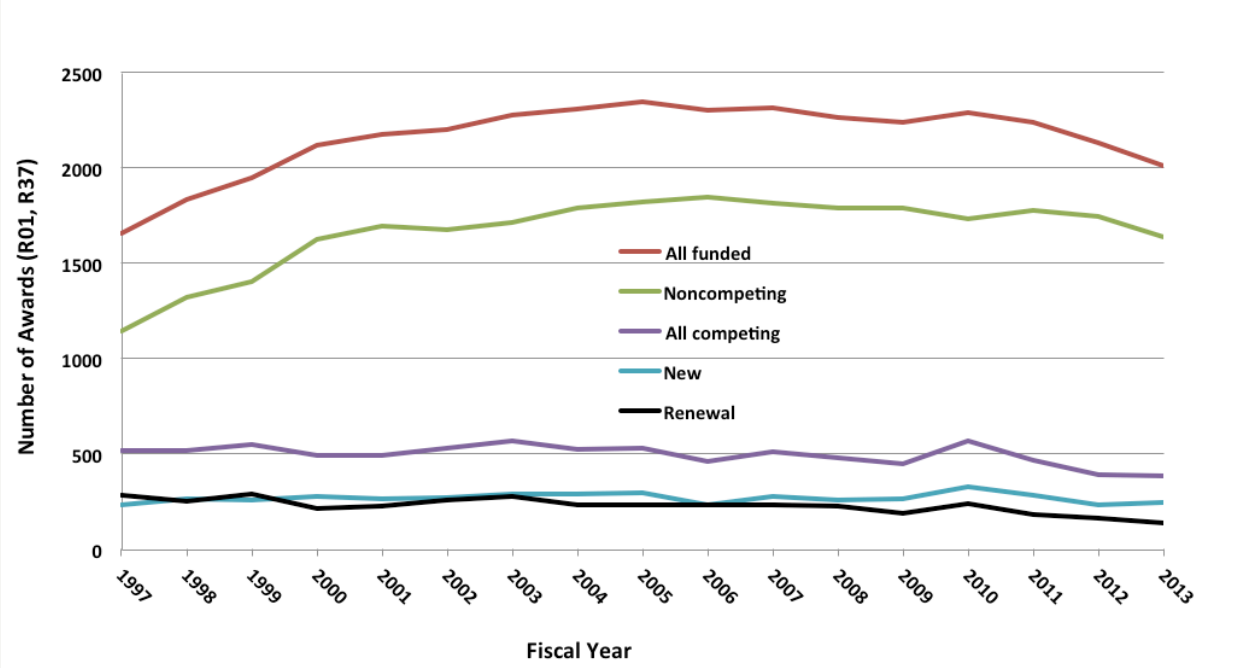
FIGURE 3: CUMULATIVE PERCENTAGE OF R01 AWARDS ACROSS PERCENTILES IN FY 2006-2013



Only funded applications are considered in the data set charted in Figure 3. Percentile bin size equals one percentile and there is no overlap between bins. Percentiles with decimal places were summed into the next highest integral percentile as follows: 0.1-0.9 was summed into 1, 1.1-1.9 was summed into 2, *etc*. These cumulative funding data again demonstrate that the vast majority of applications funded by NIDDK fall within the payline, but that NIDDK does exercise some programmatic discretion to reach for a limited number of especially innovative or programmatically important applications.

FIGURE 4

FIGURE 4: NUMBER OF NIDDK R01 AND R37 GRANTS (COMPETING AND NON-COMPETING) FUNDED IN FY 1997-2013



Overall, the total number of R01 grants funded by NIDDK has increased 22 percent since 1997. The major portion of this increase occurred during the years of the NIH budget doubling (1998-2003). Since FY 2005 there has been a decline in the number of grants funded. In general about half of the competing grants funded by NIDDK are new (Type 1) awards and half are competing renewals. However, in the past five years competing renewal awards have lagged behind new awards.

FIGURE 5

FIGURE 5: NUMBER OF COMPETING NIDDK R01 APPLICATIONS (INCLUDING REVISIONS) RECEIVED FOR FUNDING CONSIDERATION IN FY 1998-2013

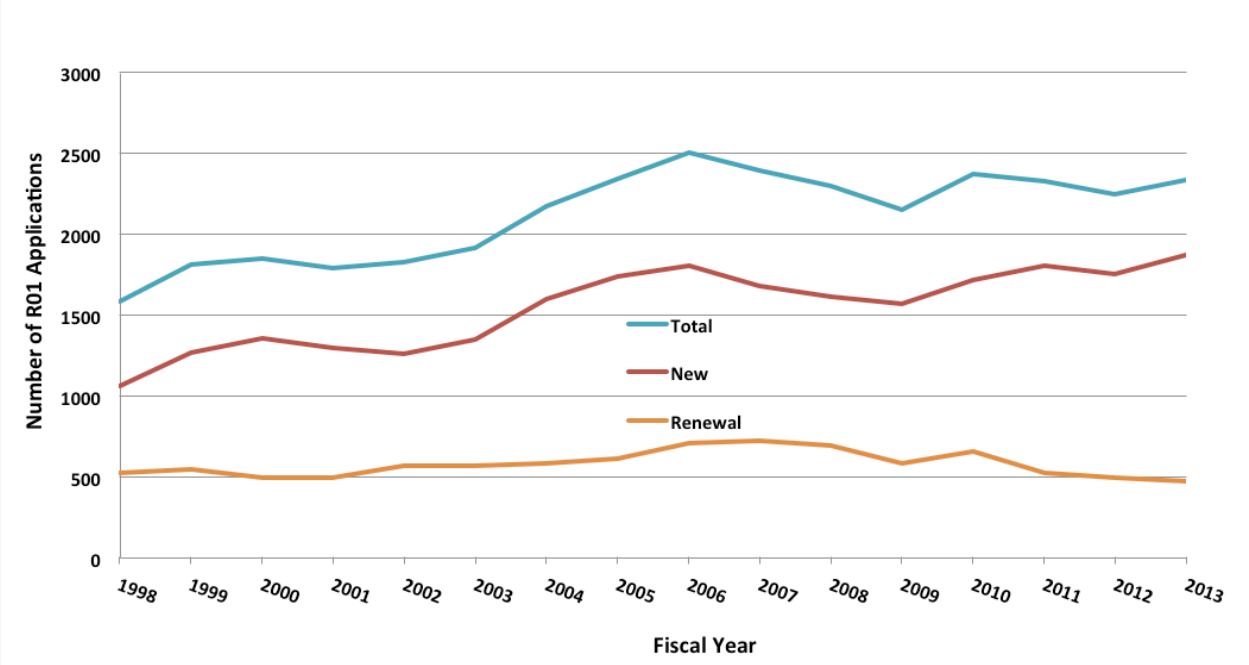


Figure 5 shows a substantial increase in the number of competing R01 applications received by NIDDK between FY 1998 and 2006. However, the numbers of competing applications have declined slightly since 2006. Much of the observed increase between 1998 and 2006 was primarily due to new (Type 1) applications. Submission rates for competing renewal applications fluctuated somewhat between 1998 and 2013, but since FY 2007 numbers of renewal applications have waned.

FIGURE 6

FIGURE 6: OVERALL NIDDK EXPENDITURES (INCLUDES DIRECT AND INDIRECT COSTS) ON R01 AWARDS (COMPETING AND NON-COMPETING) IN FY 1995-2013

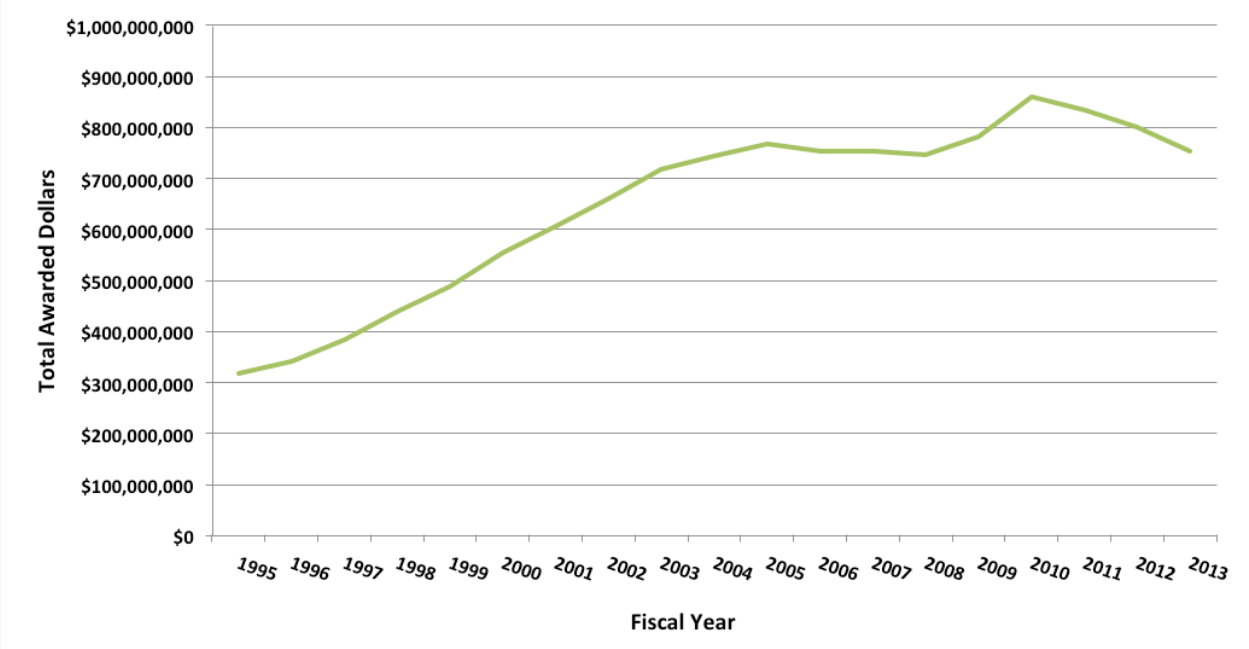


Figure 6 shows that NIDDK expenditures on R01 grants have increased markedly (137 percent) since 1995. This is because NIDDK is funding a larger number of these awards (Figure 4) and also because the median cost of an R01 has increased substantially (Figure 7).

FIGURE 7

FIGURE 7: MEDIAN TOTAL COSTS (INCLUDES DIRECT AND INDIRECT COSTS) OF NIDDK R01 GRANTS (COMPETING AND NON-COMPETING) IN FY 1995-2013

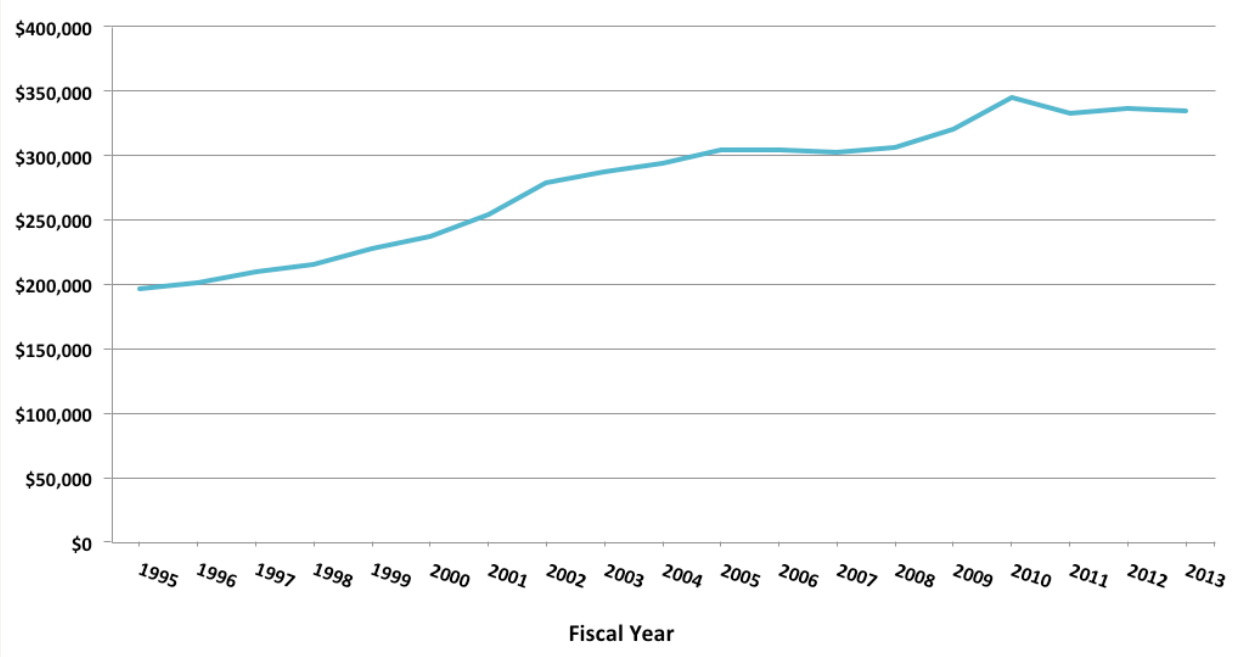
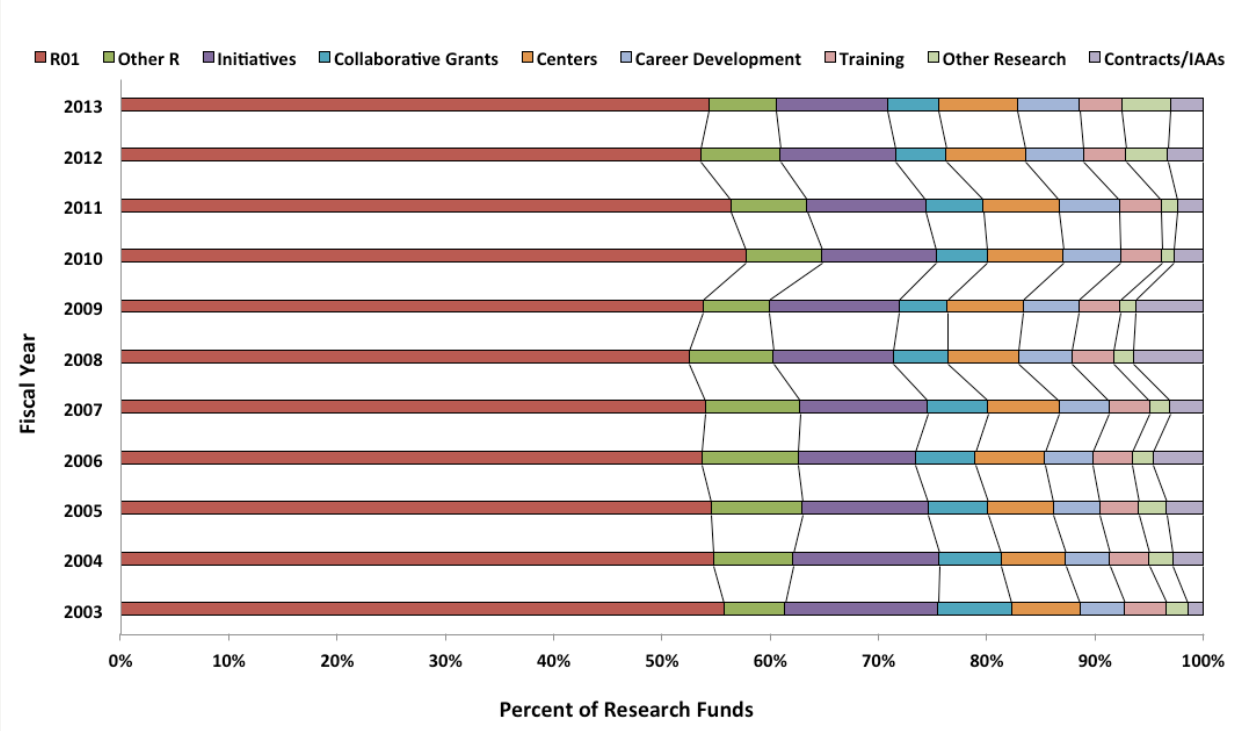


Figure 7 illustrates that the median cost of R01 awards has increased approximately 73 percent since 1995, although costs have been relatively flat since 2011. In the past 10 years (2004-2013) the number of grants receiving $500,000 or more in total costs has gone from five percent of the total number of awards to 13 percent of the total awarded R01s. The number of grants receiving $250,000 or less in total costs has declined from 25 percent of the total awards to 10 percent.

FIGURE 8

FIGURE 8: NIDDK EXTRAMURAL RESEARCH FUNDING BY CATEGORY (COMPETING AND NON-COMPETING)



NIDDK PORTFOLIO CATEGORIES:

- **R01:** Investigator initiated (excludes NIDDK RFAs)
- **Other R:** Includes other R activities (*i.e.*, R03, R13, R15, R18, R21, R34, SBIR/STTR, *etc*.) but excludes applications submitted to NIDDK RFAs and R24s
- **Initiatives:** Awards made in response to NIDDK RFAs; includes most NIDDK large clinical trials and consortia
- **Collaborative Grants:** P01s and R24s that are not "mini-Centers"
- **Centers:** Includes all non-P01 P awards and R24 "mini-Centers"
- **Career Development:** Includes all Ks (including K99/R00)
- **Training:** Includes all F and T activities
- **Other Research:** Everything not captured in the categories above
- **Contracts and Interagency Agreements (IAAs):** Includes some large clinical studies

Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2003. These data were presented to NIDDK's Advisory Council in May 2012 in the context of NIDDK's core values. The NIDDK core values emphasize maintaining a strong investigator initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials (support is generally represented in the Initiatives and Contracts categories), and continuing strong support of training and career development programs. Figures 9-12 illustrate other examples of how NIDDK's portfolio has reflected NIDDK core values over time.

FIGURE 9

FIGURE 9: PRESERVING A STABLE POOL OF NIDDK INVESTIGATORS: NUMBER OF INVESTIGATORS SUPPORTED BY AT LEAST ONE R01

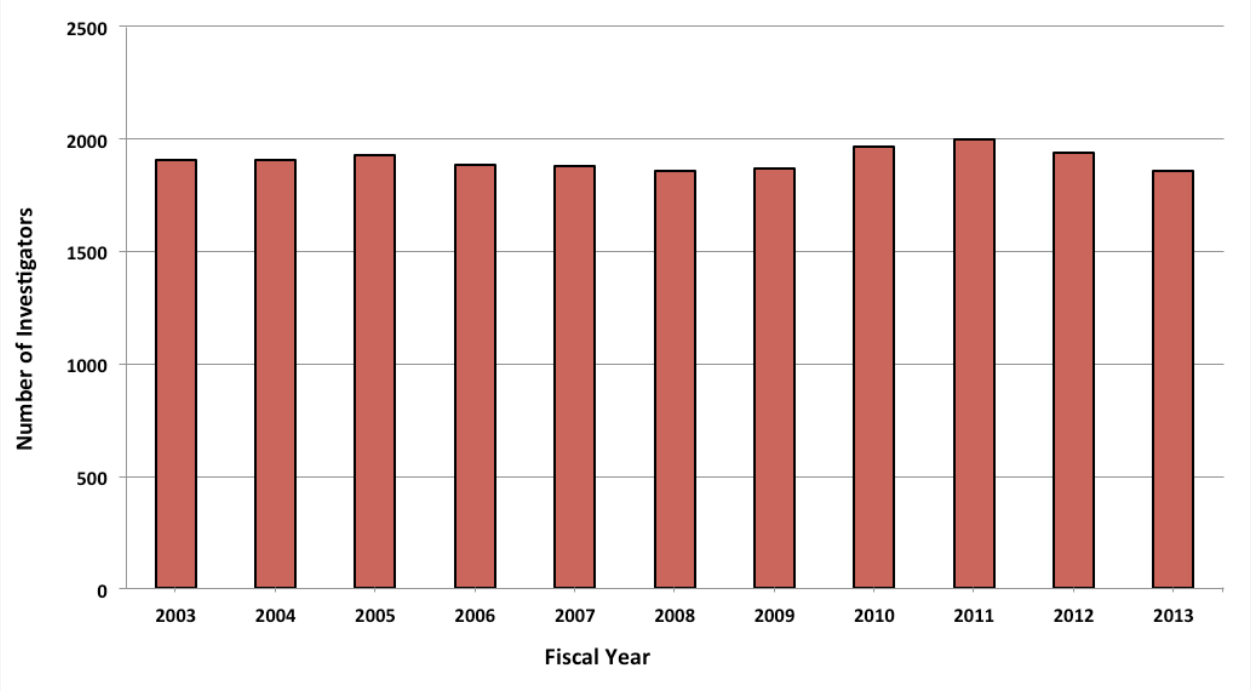


Figure 9 shows that the number of Principal Investigators (PIs) supported by at least one R01 remained relatively stable between Fiscal Year 2003 and 2009. In FYs 2010 and 2011 there were increases in the numbers of PIs supported with an NIDDK R01. It should be noted that in 2008 NIH, for the first time, began making multiple principal investigator R01 awards to support team science projects. The observed increases in numbers of PIs supported by NIDDK in FY 2010 and 2011 are largely attributable to multiple principal investigator R01 awards. The subsequent declines in 2012 and 2013 are likely due in large part because of lower paylines and inflationary pressures in the context of flat or declining budgets.

FIGURE 10

FIGURE 10: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK NEW INVESTIGATOR R01 APPLICATIONS AND AWARDS

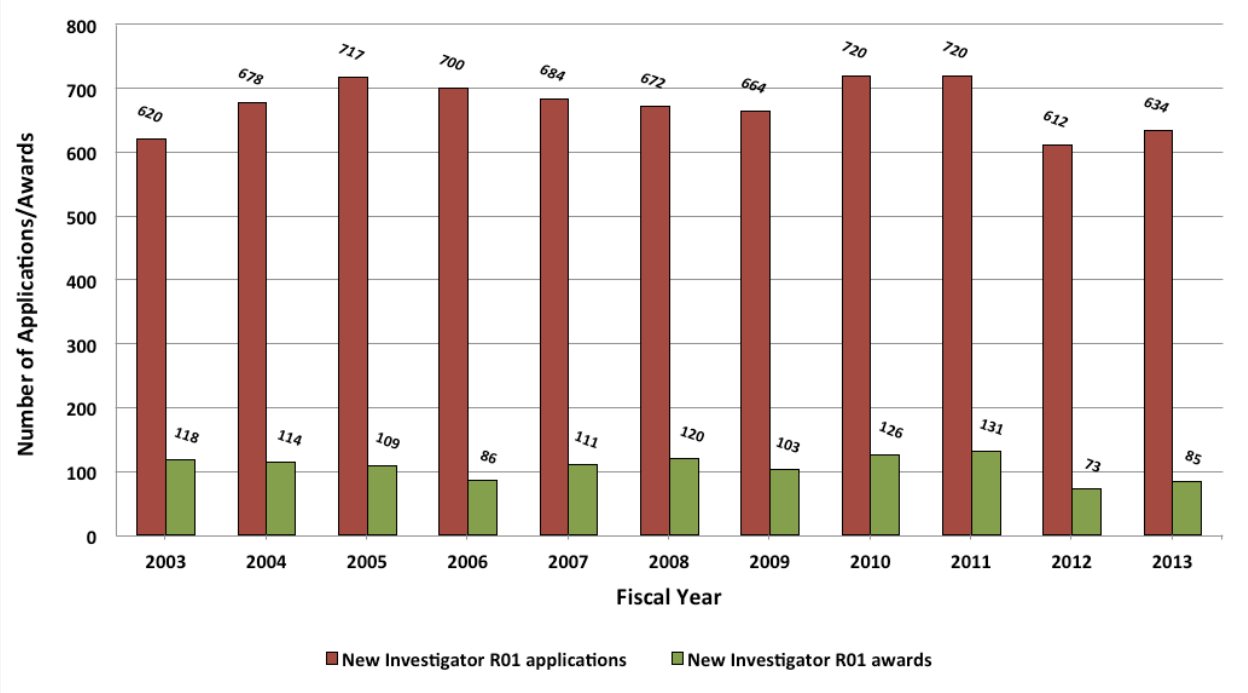
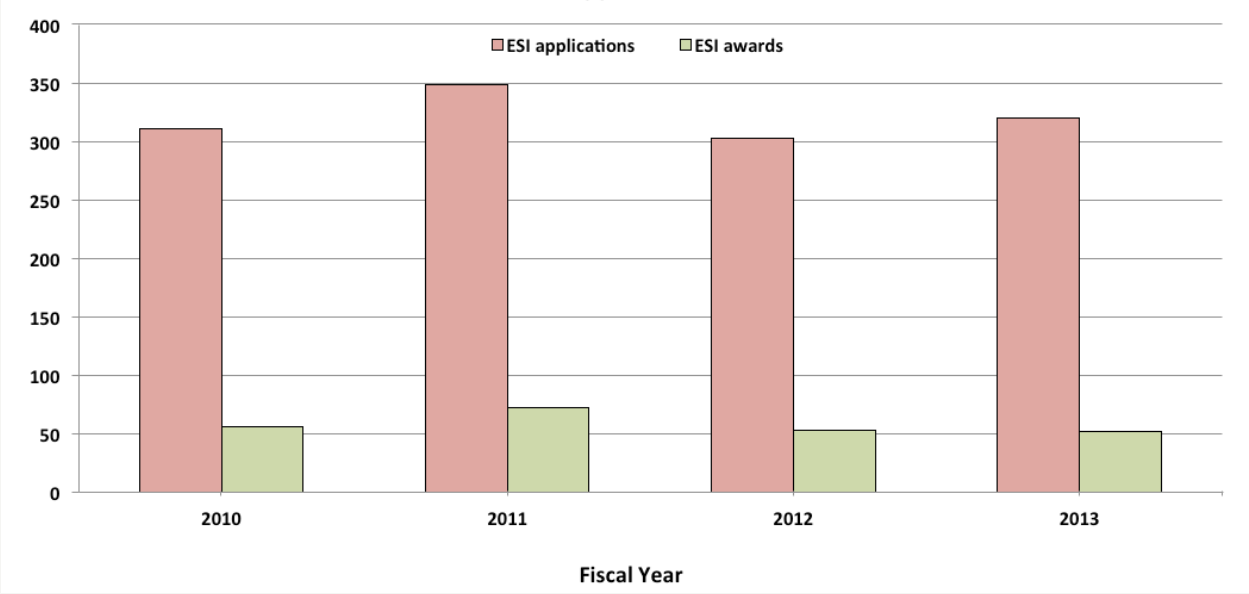


Figure 10 shows that while application rates for New Investigators have remained fairly high, there was a deceleration in the number of New Investigator awards between FY 2003 and 2006. Starting in FY 2007, NIH and NIDDK established new policies focused on New Investigators and these policies appear generally effective in mitigating downward pressures on New Investigator awards. There was, however, a sharp decrease in New Investigator applications and awards in FY 2012 with only a partial rebound in FY2013. This is a concern and NIDDK continues to carefully monitor the situation. It should be noted that in Fiscal Year 2012 NIH and NIDDK began focusing on Early Stage Investigators (ESIs), which is a subset of New Investigators (see Figures 2, 11, and 12). Counts in Figure 10 are applications and awards, not persons.

FIGURE 11

FIGURE 11: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK EARLY STAGE INVESTIGATOR (ESI) R01 APPLICATIONS AND AWARDS



Comparison of Figures 10 and 11 shows that while the subset of ESI applications fell in Fiscal Year 2012 essentially in proportion to the total drop in New Investigator applications, the proportional drop in number of awards to ESIs was not as great. This is attributable in part to NIDDK's differential payline for ESI applications. The number of ESI awards in FY 2013 was essentially flat compared with the number of ESI awards in FY 2012.

FIGURE 12

FIGURE 12: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: PERCENT OF NEW INVESTIGATOR APPLICATIONS AND AWARDS THAT ARE ESI

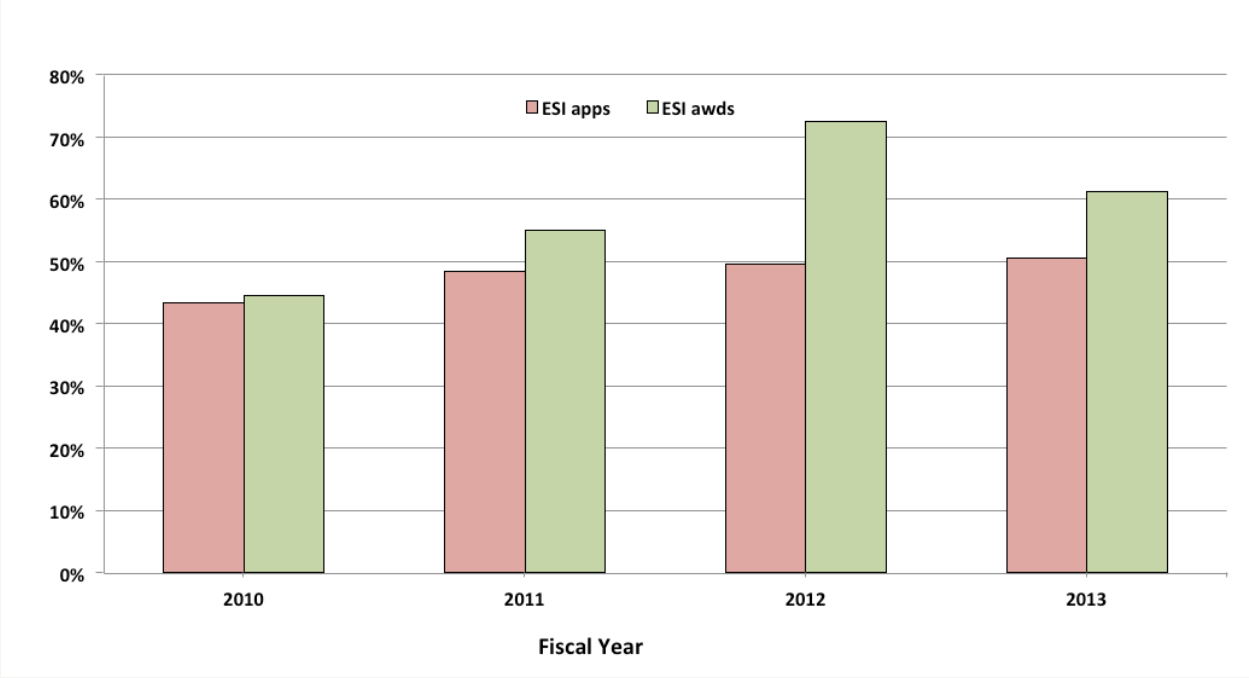


Figure 12 shows that NIDDK's differential payline for ESIs in FY 2013 was effective in continuing to enhance ESI representation among New Investigator awards.

FIGURE 13

FIGURE 13: SUPPORT PIVOTAL CLINICAL STUDIES AND TRIALS: NIDDK HUMAN SUBJECTS RESEARCH FUNDING AS A PROPORTION OF ALL EXTRAMURAL RESEARCH FUNDING

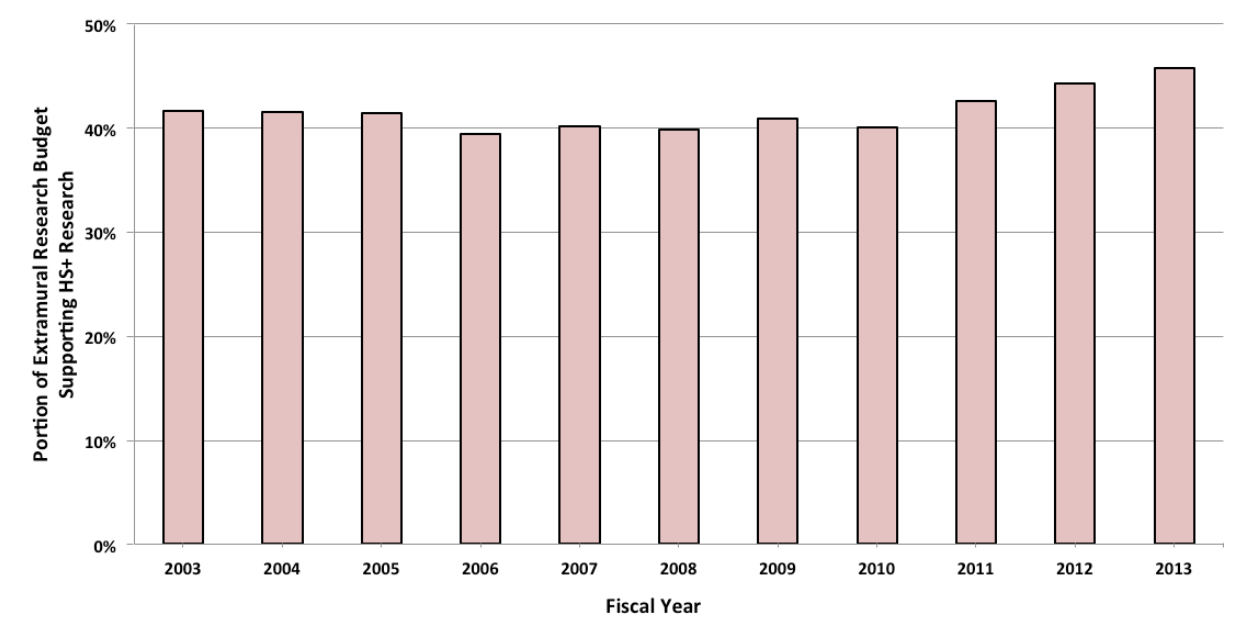


Figure 13 demonstrates that the NIDDK commits a substantial proportion of its research funding to the support of clinical research involving human subjects. For the purpose of this analysis, we used the definition described in Kotchen et al, *JAMA* 2004 Feb; 291(7):836-43 and included all studies coded as using Human Subjects (HS+).

FIGURE 14 (A TO D)

NIDDK IS COMMITTED TO TRAINING THE NEXT GENERATION

Figures 14 A to D demonstrate that NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong. Figure 14 A shows that overall support of training and career development programs has increased since 2003 and that the slight deceleration of T awards support was offset by an increase in support of F awards (by design). Figures 14 B and D illustrate that the numbers of NIDDK T awards and associated training slots have remained relatively stable. Figure 14 C shows that while the numbers of NIDDK K08 (Mentored Clinical Scientist Development Award) awards decreased between 2003 and 2013, the numbers of K01 (Mentored Research Scientist Development Award) and K23 (Mentored Patient-Oriented Research Career Development Award) have increased. NIDDK will continue to monitor carefully its training and career development programs to ensure appropriate balance.

*Please note that T32 awards made in FY 2013 continue into FY 2014. The total numbers of T32 slots are reported at the end of the award period. Therefore the FY 2013 information on T32 slots will not be available until later in FY 2014.

FIGURE 14A: NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS AS A PERCENT OF TOTAL RESEARCH FUNDING

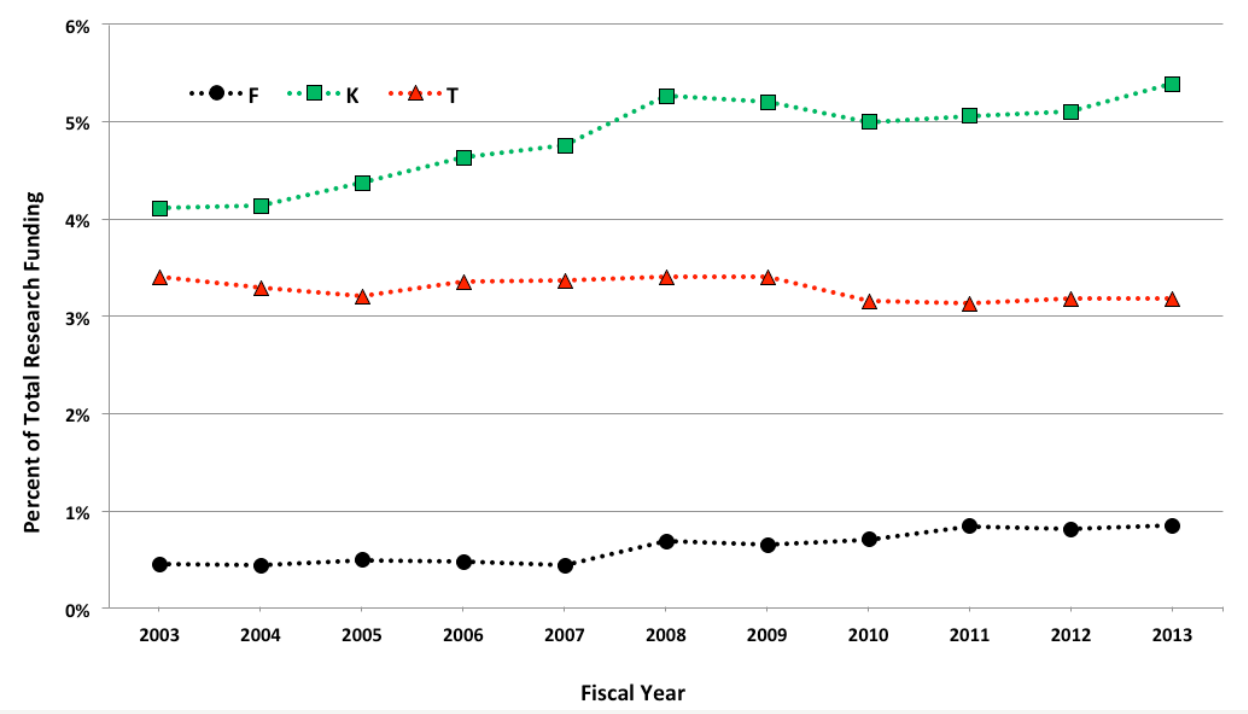


FIGURE 14B: NUMBER OF NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS BY FISCAL YEAR

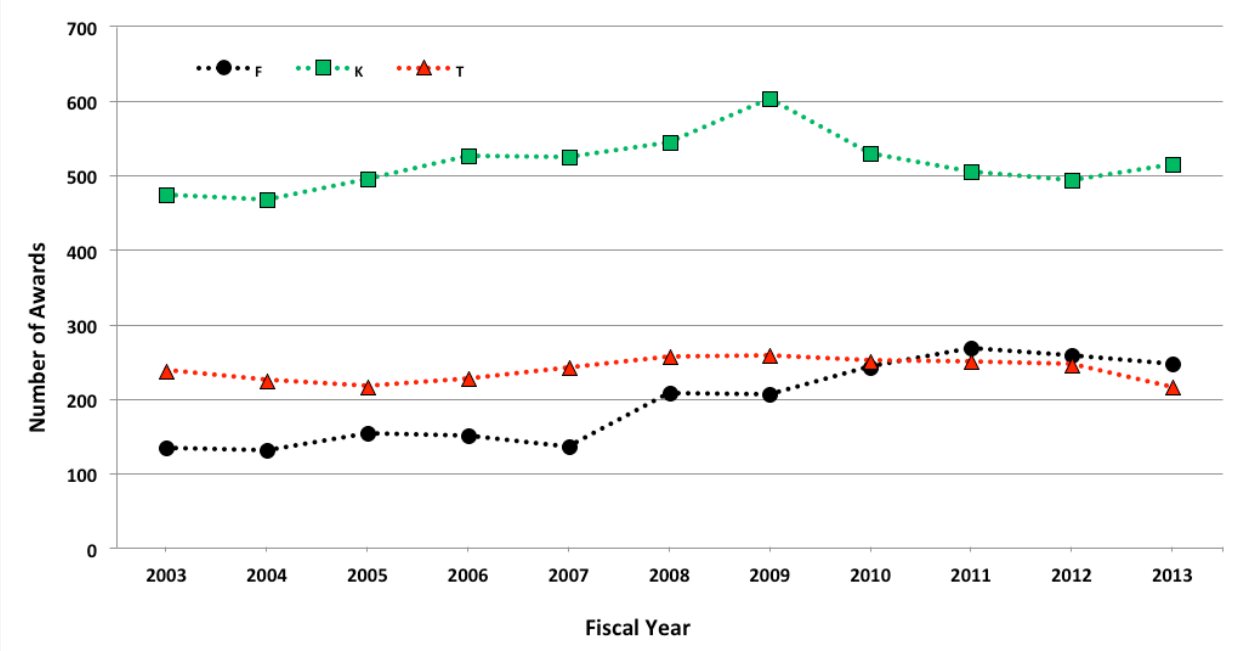


FIGURE 14C: NUMBER OF NIDDK CAREER DEVELOPMENT (K) AWARDS BY ACTIVITY AND FISCAL YEAR

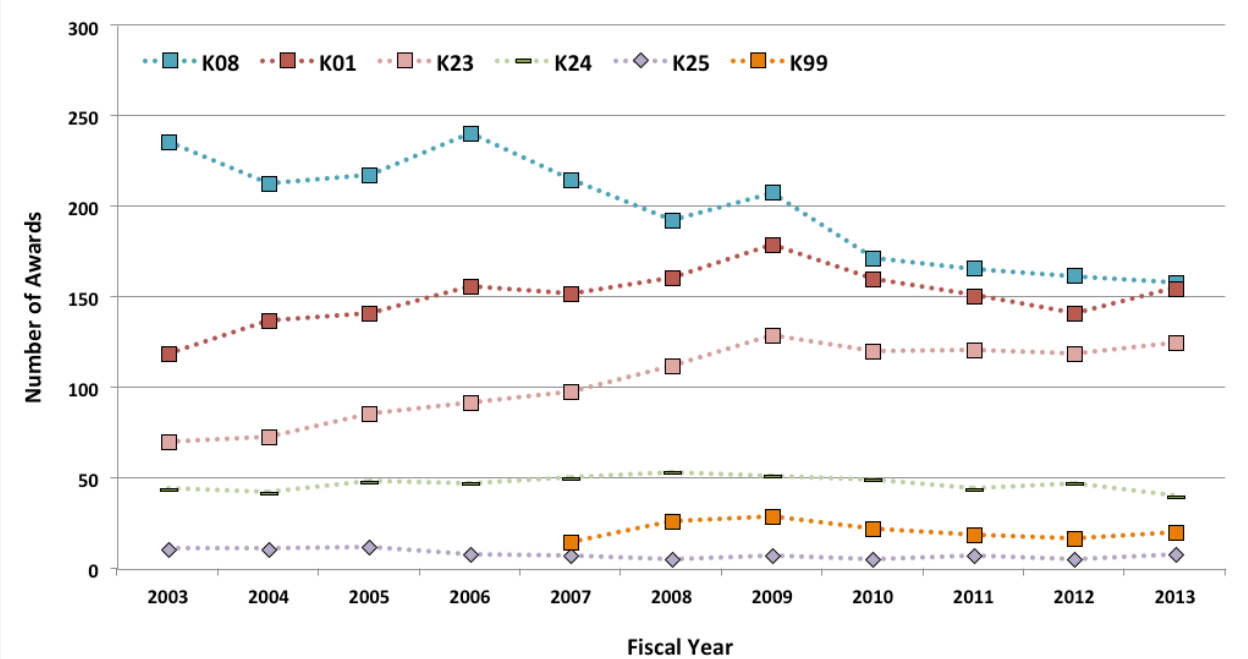
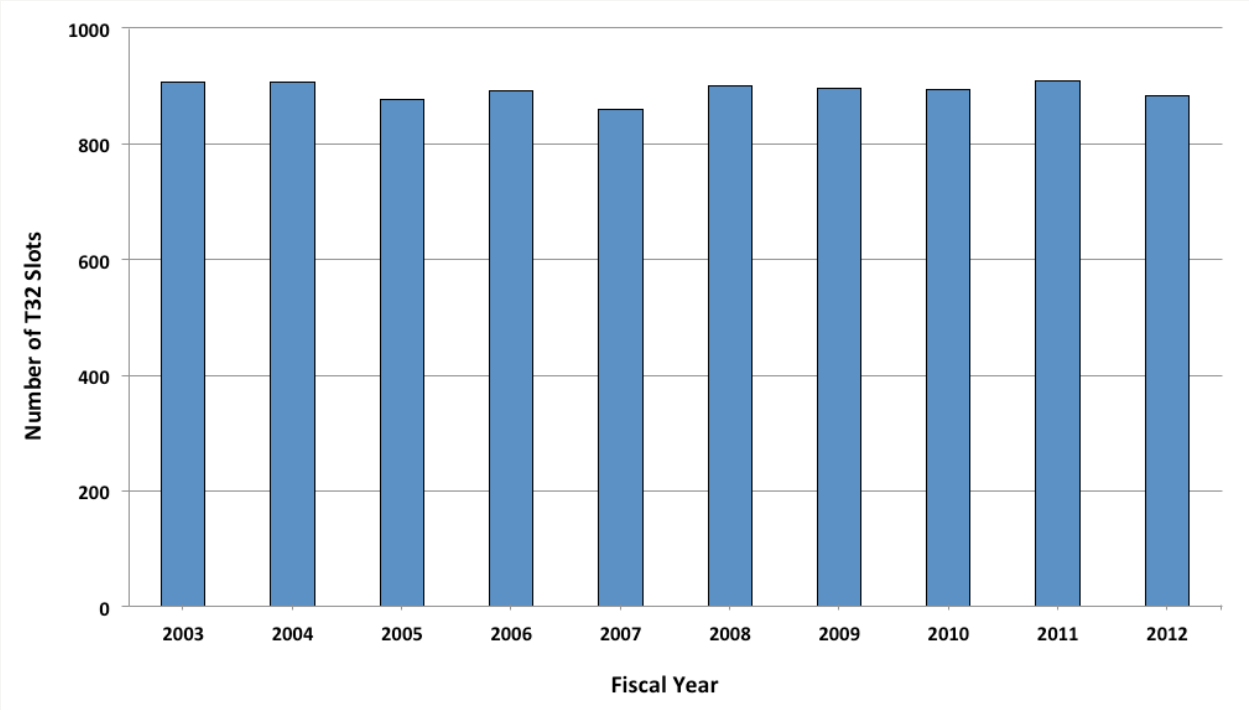


FIGURE 14D: NUMBER OF NIDDK TRAINING (T32) AWARD SLOTS BY FISCAL YEAR



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